



ELONGASE GENES AND USES THEREOF

The subject application is a Continuation-In-Part of pending U.S. patent application Serial No. 09/624,670 filed on July 24, 2000, which is a Continuation-In-Part of pending U.S. patent application Serial No. 09/379,095 filed on August 23, 1999, which is a Continuation-In-Part of pending U.S. patent application Serial No. 09/145,828 filed on September 2, 1998, all of which are herein incorporated in their entirety by reference.

BACKGROUND OF THE INVENTIONTechnical Field

The subject invention relates to the identification of several genes involved in the elongation of long-chain polyunsaturated fatty acids (i.e., "elongases") and to uses thereof. In particular, the elongase enzyme is utilized in the conversion of one fatty acid to another. For example, elongase catalyzes the conversion of gamma linolenic acid (GLA) to dihomo- $\gamma$ -linolenic acid (DGLA, 20:3n-6) and the conversion of stearidonic acid (STA, 18:4n-3) to (n-3)-eicosatetraenoic acid (20:4n-3). Elongase also catalyzes the conversion of arachidonic acid (AA, 20:4n-6) to adrenic acid (ADA, 22:4n-6), the conversion of eicosapentaenoic acid (EPA, 20:5n-3) to  $\omega$ 3-docosapentaenoic acid (22:5n-3), and the conversion of  $\alpha$ -linolenic acid (ALA, 18:3n-3) to 20:3n-3. DGLA, for example, may be utilized in the production of other polyunsaturated fatty acids (PUFAs), such as arachidonic acid (AA) which may be added to pharmaceutical compositions, nutritional compositions, animal feeds, as well as other products such as cosmetics.

Background Information

The elongases which have been identified in the past differ in terms of the substrates upon which they act. Furthermore, they are present in both animals and plants. Those found in mammals have the ability to act on saturated, monounsaturated and polyunsaturated fatty acids. In contrast, those found in plants are specific for saturated or monounsaturated fatty acids. Thus, in order to generate polyunsaturated fatty acids in plants, there is a need for a PUFA-specific elongase.

In both plants and animals, the elongation process is believed to be the result of a four-step mechanism (Lassner et al., *The Plant Cell* 8:281-292 (1996)). CoA is the acyl carrier. Step one involves condensation of malonyl-CoA with a long-chain acyl-CoA to yield carbon dioxide and a  $\beta$ -ketoacyl-CoA in which the acyl moiety has been elongated by two carbon atoms. Subsequent reactions include reduction to  $\beta$ -hydroxyacyl-CoA; dehydration to an enoyl-CoA, and a second reduction to yield the elongated acyl-CoA. The initial condensation reaction is not only the substrate-specific step but also the rate-limiting step.

As noted previously, elongases, more specifically, those which utilize PUFAs as substrates, are critical in the production of long-chain polyunsaturated fatty acids which have many important functions. For example, PUFAs are important components of the plasma membrane of a cell where they are found in the form of phospholipids. They also serve as precursors to mammalian prostacyclins, eicosanoids, leukotrienes and prostaglandins. Additionally, PUFAs are necessary for the proper development of the developing infant brain as well as for tissue formation and repair. In view of the biological

significance of PUFAs, attempts are being made to produce them, as well as intermediates leading to their production, efficiently.

A number of enzymes are involved in PUFA biosynthesis including elongases (el) (see Figure 1). For example, linoleic acid (LA, 18:2- $\Delta$ 9,12 or 18:2n-6) is produced from oleic acid (OA, 18:1- $\Delta$ 9 or 18:1n-9) by a  $\Delta$ 12 desaturase. GLA (18:3- $\Delta$ 6,9,12) is produced from linoleic acid by a  $\Delta$ 6-desaturase. AA (20:4- $\Delta$ 5,8,11,14) is produced from dihomoy-linolenic acid (DGLA, 10:20:3- $\Delta$ 8,11,14) by a  $\Delta$ 5-desaturase. As noted above, DGLA is produced from GLA by an elongase.

It must be noted that animals cannot desaturate beyond the  $\Delta$ 9 position and therefore cannot convert oleic acid into linoleic acid. Likewise,  $\alpha$ -linolenic acid (ALA, 18:3- $\Delta$ 9,12,15 or 18:3n-3) cannot be synthesized by mammals, since they lack  $\Delta$ 15 desaturase activity. However,  $\alpha$ -linolenic acid can be converted to stearidonic acid (STA, 18:4- $\Delta$ 6,9,12,15) by a  $\Delta$ 6-desaturase (see PCT publication WO 96/15591; see also U.S. Patent No. 5,552,306), followed by elongation to (n-3)-eicosatetraenoic acid (20:4- $\Delta$ 8,11,14,17 or 20:4n-3) in mammals and algae. This polyunsaturated fatty acid (i.e., 20:4- $\Delta$ 8,11,14,17) can then be converted to eicosapentaenoic acid (EPA, 20:5- $\Delta$ 5,8,11,14,17) by a  $\Delta$ 5-desaturase. Other eukaryotes, including fungi and plants, have enzymes which desaturate at carbons 12 (see PCT publication WO 94/11516 and U.S. Patent No. 5,443,971) and 15 (see PCT publication WO 93/11245). The major polyunsaturated fatty acids of animals therefore are either derived from diet and/or from desaturation and elongation of linoleic acid or  $\alpha$ -linolenic acid. In view of the inability of mammals to produce these essential long chain fatty acids, it is of significant interest to isolate

genes involved in PUFA biosynthesis from species that naturally produce these fatty acids and to express these genes in a microbial, plant or animal system which can be altered to provide production of commercial quantities of one or more PUFAs. Consequently, there is a definite need for the elongase enzyme, the gene encoding the enzyme, as well as recombinant methods of producing this enzyme. Additionally, a need exists for oils containing levels of PUFA beyond those naturally present as well as those enriched in novel PUFAs. Such oils can only be made by isolation and expression of the elongase gene.

One of the most important long chain PUFAs, noted above, is arachidonic acid (AA). AA is found in filamentous fungi and can also be purified from mammalian tissues including the liver and the adrenal glands. As noted above, AA production from DGLA is catalyzed by a  $\Delta$ 5-desaturase, and DGLA production from  $\gamma$ -linolenic acid (GLA) is catalyzed by an elongase. However, until the present invention, no elongase had been identified which was active on substrate fatty acids in the pathways for the production of long chain PUFAs and, in particular, AA, eicosapentaenoic acid (EPA), aridenic acid, docosahexaenoic acid (DHA, 22:6n-3),  $\omega$ 3-docosapentaenoic acid (22:5n-3) or  $\omega$ 6-docosapentaenoic acid (22:5n-6).

Two genes appeared to be of interest in the present search for the elongase gene. In particular, the jojoba  $\beta$ -ketacyl-1-coenzyme A synthase (KCS), or jojoba KCS (GenBank Accession # U37088), catalyzes the initial reaction of the fatty acyl-CoA elongation pathway (i.e., the condensation of malonyl-CoA with long-chain acyl-CoA (Lassner et al., *The Plant Cell* 8:281-292 (1996)). Jojoba KCS substrate preference is 18:0, 20:0, 20:1, 30:1, 22:1, 22:0 and 16:0. Saccharomyces cerevisiae elongase

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(ELO2) also catalyzes the conversion of long chain saturated and monounsaturated fatty acids, producing high levels of 22:0, 24:0, and also 18:0, 18:1, 20:0, 20:1, 22:0, 22:1, and 24:1 (Oh et al., The Journal of Biological Chemistry 272 (28):17376-17384 (1997); see also U.S. Patent No. 5,484,724 for a nucleotide sequence which includes the sequence of ELO2; see PCT publication WO 88/07577 for a discussion of the sequence of a glycosylation inhibiting factor which is described in Example V). The search for a long chain PUFA-specific elongase in Mortierella alpina began based upon a review of the homologies shared between these two genes and by expression screening for PUFA-elongase activity.

#### SUMMARY OF THE INVENTION

15 The present invention relates to an isolated nucleotide sequence corresponding to or complementary to at least about 50% of the nucleotide sequence shown in SEQ ID NO:1 (Figure 6). This isolated sequence may be represented by SEQ ID NO:1. The sequence encodes a functionally active elongase which utilizes a 20 polyunsaturated fatty acid or a monounsaturated fatty acid as a substrate. In particular, the sequence may be derived from a fungus of the genus Mortierella and may specifically be isolated from Mortierella alpina.

The present invention also includes a purified protein 25 encoded by the above nucleotide sequence as well as a purified polypeptide which elongates polyunsaturated fatty acids or monounsaturated fatty acids and has at least about 50% amino acid similarity to the amino acid sequence of the purified protein encoded by the above nucleotide sequence.

30 Additionally, the present invention encompasses a method of producing an elongase enzyme comprising the steps of: a)

isolating the nucleotide sequence represented by SEQ ID NO:1 (Figure 6); b) constructing a vector comprising: 1) the isolated nucleotide sequence operably linked to ii) a promoter; and c) introducing the vector into a host cell under time and

5 conditions sufficient for expression of the elongase enzyme. The host cell may be a eukaryotic cell or a prokaryotic cell.

The prokaryotic cell may be, for example an E. coli cell, a cyanobacterial cell, or a B. subtilis cell. The eukaryotic cell may be, for example, a mammalian cell, an insect cell, a plant cell or a fungal cell. The fungal cell may be, for example, Saccharomyces spp., Candida spp., Lipomyces spp., Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. or Pichia spp.

15 Saccharomyces spp., in particular, Saccharomyces cerevisiae, Candida spp., Hansenula spp. or Pichia spp. The invention also includes a vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:1 (Figure 6) operably linked to b) a promoter, as well as a host cell

20 comprising this vector. The host may be a prokaryotic cell or a eukaryotic cell. Suitable examples of prokaryotic cells include E. coli, Cyanobacteria, and B. subtilis cells. Suitable examples of eukaryotic cells include a mammalian cell, an insect cell, a plant cell and a fungal cell. The fungal cell may be, for example, Saccharomyces spp., Candida spp., Lipomyces spp., Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. and Pichia spp. In particular, the fungal cell may be, for example, a yeast cell such as, for example, Saccharomyces spp., in 30 particular, Saccharomyces cerevisiae, Candida spp., Hansenula spp. and Pichia spp.

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The present invention includes a plant cell, plant or plant tissue comprising the above-described vector, wherein expression of the nucleotide sequence of the vector results in production of at least one fatty acid selected from the group consisting of a monounsaturated fatty acid and a polyunsaturated fatty acid by the plant cell, plant or plant tissue. The polyunsaturated fatty acid may be, for example, dihomo- $\gamma$ -linolenic acid (DGLA), 20:4n-3, and arachidonic acid (ADA). The invention also includes one or more plant oils or fatty acids expressed by the plant cell, plant or plant tissue. Additionally, the present invention encompasses a transgenic plant comprising the above-described vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in seeds of the transgenic plant.

Furthermore, the present invention includes a transgenic, non-human mammal whose genome comprises a DNA sequence encoding an elongase operably linked to a promoter. The DNA sequence may be represented by SEQ ID NO:1 (Figure 6). The present invention also includes a fluid (e.g., milk) produced by the transgenic, non-human wherein the fluid comprises a detectable level of at least one elongase or products thereof such as, for example, DGLA,  $\omega$ 6-docosapentaenoic acid, ADA and/or 20:4n-3 (see Figure 1).

Additionally, the present invention includes a method for producing a polyunsaturated fatty acid comprising the steps of:

- isolating said nucleotide sequence represented by SEQ ID NO:1 (Figure 6);
- constructing a vector comprising the isolated nucleotide sequence;
- introducing the vector into a host cell under time and conditions sufficient for expression of elongase enzyme encoded by the isolated nucleotide sequence; and
- exposing the expressed elongase enzyme to a "substrate"

polyunsaturated fatty acid in order to convert the substrate to a "product" polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be selected from the group consisting of, for example,  $\gamma$ -linolenic acid (GLA), stearidonic acid (STA) and arachidonic acid (AA), and the product polyunsaturated fatty acid may be selected from the group consisting of, for example, DGLA, 20:4n-3, and ADA, respectively. The method may further comprise the step of exposing the product polyunsaturated fatty acid to at least one desaturase in order to convert the product polyunsaturated fatty acid to "another" polyunsaturated fatty acid. The product polyunsaturated fatty acid may be selected from the group consisting of, for example, DGLA, 20:4n-3, and ADA. The another polyunsaturated fatty acid may be selected from the group consisting of, for example, AA, eicosapentaenoic acid (EPA),  $\omega$ 6-docosapentaenoic acid, respectively, and the at least one desaturase is  $\Delta$ 5-desaturase, with respect to production of AA or EPA, and  $\Delta$ 4-desaturase, with respect to production of  $\omega$ 6-docosapentaenoic acid. The method may further comprise the step of exposing the another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert the another polyunsaturated fatty acid to a "final" polyunsaturated fatty acid. The final polyunsaturated fatty acid may be, for example, docosahexaenoic acid (DHA), AA,  $\omega$ 6-docosapentaenoic acid, or  $\omega$ 3-docosapentaenoic acid.

Also, the present invention includes a nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-

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described method, the another polyunsaturated fatty acid produced according to the above-described method, and the final polyunsaturated fatty acid produced according to the above-described method. The product polyunsaturated fatty acid may be selected from the group consisting of, for example, DGLA, 20:4n-3 and ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA, or  $\omega$ 6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, adrenic acid,  $\omega$ 6-docosapentaenoic acid or  $\omega$ 3-docosapentaenoic acid.

The nutritional composition may be, for example, an infant formula, a dietary supplement or a dietary substitute and may be administered to a human or an animal and may be administered enterally or parenterally. The nutritional composition may further comprise at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, monoglycerides, triglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, protein hydrolysates, sunflower oil, safflower oil, corn oil, and flax oil. It may also comprise at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex and at least one mineral selected from the group consisting of calcium, magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium and iron.

Additionally, the present invention encompasses a pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another polyunsaturated fatty acid produced according to the above-described method, and the final

polyunsaturated fatty acid produced according to the above-described method and 2) a pharmaceutically acceptable carrier. The composition may be administered to a human or an animal. It may also further comprise at least one element selected from the group consisting of a vitamin, a mineral, a salt, a carbohydrate, an amino acid, a free fatty acid, a preservative, an excipient, an anti-histamine, a growth factor, an antibiotic, a diluent, a phospholipid, an antioxidant, and a phenolic compound. It may be administered enterally, parenterally, topically, rectally, intramuscularly, subcutaneously, intradermally, or by any other appropriate means.

The present invention also includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another polyunsaturated fatty acid produced according to the above-described method, and the final polyunsaturated fatty acid produced according to the above-described method. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, AA, EPA, or  $\omega$ 6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, adrenic acid,  $\omega$ 6-docosapentaenoic acid or  $\omega$ 3-docosapentaenoic acid. Moreover, the present invention also includes a cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another polyunsaturated fatty acid produced according to the above-described method, and the final polyunsaturated fatty acid produced according to the above-described method.

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Additionally, the present invention includes a method of preventing or treating a condition caused by insufficient intake or production of polyunsaturated fatty acids comprising administering to the patient the above nutritional composition in an amount sufficient to effect prevention or treatment.

The present invention also includes an isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence shown in SEQ ID NO:2 (Figure 22). This sequence may be represented by SEQ ID NO:2. The sequence encodes a functionally active elongase which utilizes a polyunsaturated fatty acid as a substrate. This sequence may also be derived, for example, from a fungus of the genus Mortierella. In particular, it may be derived from M. alpina.

Additionally, the present invention includes a purified protein encoded by the above nucleotide sequence as well as a purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of the purified protein.

The present invention also includes a method of producing an elongase enzyme as described above. The sequence inserted in the vector is represented by SEQ ID NO:2 (Figure 22). The host cell may be prokaryotic or eukaryotic. Suitable examples are described above.

The present invention also includes a vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:2 (Figure 22) operably linked to b) a promoter, as well as a host cell comprising this vector. Again, the host cell may be eukaryotic or prokaryotic. Suitable examples are described above.

The invention also includes a plant cell, plant or plant tissue comprising the above vector, wherein expression of the nucleotide sequence of the vector results in production of a

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polyunsaturated fatty acid by the plant cell, plant or plant tissue. The polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA. Additionally, the invention includes one or more plant oils or fatty acids expressed by the plant cell, plant or plant tissue.

Furthermore, the present invention also includes a transgenic plant comprising the above vector, wherein expression of the nucleotide sequence (SEQ ID NO:2) of the vector results in production of a polyunsaturated fatty acid in seeds of the plant or plant tissue.

The invention also includes a transgenic, non-human mammal whose genome comprises a DNA sequence (SEQ ID NO:2) encoding an elongase operably linked to a promoter. The invention also includes a fluid produced by this transgenic, non-human mammal wherein the fluid comprises a detectable level of at least one elongase or products thereof.

The present invention also includes a method for producing a polyunsaturated fatty acid comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:2 (Figure 22); b) constructing a vector comprising the isolated nucleotide sequence; c) introducing the vector into a host cell under time and conditions sufficient for expression of an elongase enzyme encoded by the isolated nucleotide sequence; and d) exposing the expressed elongase enzyme to a substrate.

20 The method may further comprise the step of exposing the product polyunsaturated fatty acid to convert the substrate to a product polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be, for example, GIA, STA, or AA, the product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or  $\omega$ 6-docosapentaenoic acid, respectively.

25 The method may further comprise the step of exposing the expressed elongase enzyme to at least one desaturase in order to

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convert the product polyunsaturated fatty acid to another polyunsaturated fatty acid. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA, the another polyunsaturated fatty acid may be, for example, AA, EPA, or  $\omega$ 6-docosapentaenoic acid, respectively, and the at least one desaturase is  $\Delta$ 5-desaturase with respect to production of AA or EPA, and  $\Delta$ 4-desaturase with respect to production of  $\omega$ 6-docosapentaenoic acid. The method may further comprise the step of exposing the another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert the another polyunsaturated fatty acid to a final polyunsaturated fatty acid. The final polyunsaturated fatty acid may be, for example, docosahexaenoic acid, AA,  $\omega$ 6-docosapentaenoic acid, or  $\omega$ 3-docosapentaenoic acid.

The invention also includes a nutritional composition comprising at least one polyunsaturated fatty acid selected from the product polyunsaturated fatty acid produced according to the method described with respect to SEQ ID NO:2, the another polyunsaturated fatty acid produced according to the method described with respect to SEQ ID NO:2, and the final polyunsaturated fatty acid produced according to the method described with respect to SEQ ID NO:2. The product polyunsaturated fatty acid may be selected from the group consisting of, for example, DGLA, 20:4n-3 and ADA. The another polyunsaturated fatty acid may be selected from the group consisting of, for example, AA, EPA, and  $\omega$ 6-docosapentaenoic acid. The final polyunsaturated fatty acid may be selected from the group consisting of, for example, DHA, AA,  $\omega$ 6-docosapentaenoic acid, and  $\omega$ 3-docosapentaenoic acid.

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The other attributes of the composition are the same as those described above with respect to administration, characterization, components, etc.

The present invention also includes a pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the method of noted above with respect to SEQ ID NO:2, the another polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2, and the final polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2, and 2) a pharmaceutically acceptable carrier. The characteristics of the above-described pharmaceutical composition (e.g., administration, components, etc.) also apply to this composition.

The present invention also includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method described with respect to SEQ ID NO:2, the another polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2, and the final polyunsaturated fatty acid produced according to the method described with respect to SEQ ID NO:2. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3 or ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA or  $\omega$ 6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, adrenic acid,  $\omega$ 6-docosapentaenoic acid or  $\omega$ 3-docosapentaenoic acid.

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The invention also includes a cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2, the another polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2, and the final polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2.

Additionally, the present invention includes a method of preventing or treating a condition caused by insufficient intake or production of polyunsaturated fatty acids comprising administering to the patient the nutritional composition described directly above in an amount sufficient to effect the prevention or treatment.

Furthermore, the present invention includes an isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence shown in SEQ ID NO:3 (Figure 43). This sequence may be that represented by SEQ ID NO:3. This sequence encodes a functionally active elongase which utilizes a polyunsaturated fatty acid or a monounsaturated fatty acid as a substrate. The sequence is derived from a mammal such as, for example, a human.

The invention also includes a purified protein encoded by this nucleotide sequence. Also, the invention includes a purified polypeptide which elongates polyunsaturated fatty acids or monounsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of this purified protein.

Additionally, the invention includes method of producing an elongase enzyme comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:3 (Figure 43); b)

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constructing a vector comprising: i) the isolated nucleotide sequence operably linked to ii) a promoter; and c) introducing said vector into a host cell under time and conditions sufficient for expression of the elongase enzyme. The host cell 5 may be the same as that described above with respect to the corresponding methods utilizing SEQ ID NO:1 or 2.

The invention also includes a vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:3 (Figure 43) operably linked to b) a promoter, as well as a host cell 10 comprising this vector. The host cell may be the same as that described above.

The invention also includes a plant cell, plant or plant tissue comprising the above-described vector comprising SEQ ID NO:3, wherein expression of the nucleotide sequence of the 15 vector results in production of at least one fatty acid selected from the group consisting of a monounsaturated fatty acid and a polyunsaturated fatty acid by said plant cell, plant or plant tissue. The polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3 or ADA. The invention also includes one or more 20 plant oils or acids expressed by the plant cell, plant or plant tissue.

The invention also includes a transgenic plant comprising the vector comprising SEQ ID NO:3, wherein expression of the nucleotide sequence of the vector results in production of a 25 polyunsaturated fatty acid in seeds of the transgenic plant. Additionally, the present invention includes a transgenic, non-human mammal whose genome comprises a human DNA sequence encoding an elongase operably linked to a promoter. The DNA sequence is represented by SEQ ID NO:3 (Figure 43). The 30 invention also includes a fluid produced by said transgenic,

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non-human mammal wherein said fluid comprises a detectable level of at least one elongase or products thereof.

The invention also encompasses a method for producing a polyunsaturated fatty acid comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:3 (Figure 43); b) constructing a vector comprising said nucleotide sequence; c) introducing the vector into a host cell under time and conditions sufficient for expression of elongase enzyme encoded by the isolated nucleotide sequence; and d) exposing the expressed elongase enzyme to a substrate polyunsaturated fatty acid in order to convert the substrate to a product polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be, for example, GLA, STA or AA, and the product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA, respectively. The method may further comprise the step of exposing the product polyunsaturated fatty acid to at least one desaturase in order to convert the product polyunsaturated fatty acid to another polyunsaturated fatty acid. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3 and ADA, the another polyunsaturated fatty acid may be, for example, AA, EPA, and  $\omega$ 6-docosapentaenoic acid, respectively, and the at least one desaturase is  $\Delta$ 5-desaturase with respect to production of AA or EPA and  $\Delta$ 4-desaturase with respect to production of  $\omega$ 6-docosapentaenoic acid. The method may further comprise the step of exposing the another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert the another polyunsaturated fatty acid to a final polyunsaturated fatty acid. The final polyunsaturated

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fatty acid may be, for example, DHA, ADA,  $\omega$ 6-docosapentaenoic acid, and  $\omega$ 3-docosapentaenoic acid.

The nutritional composition comprising at least one polyunsaturated fatty acid which may be, for example, product 5 polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:3, another polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:3, and the final polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:3. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA. The another polyunsaturated fatty acid may be selected from the group consisting of AA, EPA, or  $\omega$ 6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, 10 DHA, ADA,  $\omega$ 6-docosapentaenoic acid, or  $\omega$ 3-docosapentaenoic acid. The other properties or characteristic of the nutritional 15 composition (e.g., administration, components, etc.) as the same as those recited above with respect to the other nutritional composition.

Moreover, the present invention also includes a pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:3, the 20 another polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:3, and the final polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:3 and 2) a pharmaceutically acceptable carrier. The other properties of 25 the composition (e.g., administration, additional components,

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etc.) are the same as those recited above with respect to the other pharmaceutical compositions.

The present invention also includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method recited above with respect to SEQ ID NO:3, the another polyunsaturated fatty acid produced according to the method recited above with respect to SEQ ID NO:3, and the final polyunsaturated fatty acid produced according to the method recited above with respect to SEQ ID NO:3. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA. The polyunsaturated fatty acid may be, for example, AA, EPA, or ω6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, ADA, ω6-docosapentaenoic acid or ω3-docosapentaenoic acid.

Also, the present invention includes a cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method recited above with respect to SEQ ID NO:3, said another polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:3, and the final polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:3.

A method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to the patient the nutritional composition recited above in connection with SEQ ID NO:3 in an amount sufficient to effect the prevention or treatment.

Additionally, the present invention includes an isolated nucleotide sequence corresponding to or complementary to at

least about 35% of the nucleotide sequence shown in SEQ ID NO:4 (Figure 46). The sequence may be represented by SEQ ID NO:4. It encodes a functionally active elongase which utilizes a polyunsaturated fatty acid as a substrate. The sequence may be derived or isolated from a nematode of the genus Caenorhabditis and, in particular, may be isolated from C. elegans.

The present invention includes a purified protein encoded by the nucleotide sequence above. The invention also includes a purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of the purified protein.

Additionally, the present invention includes a method of producing an elongase enzyme comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:4 (Figure 46); b) constructing a vector comprising: 1) the isolated nucleotide sequence operably linked to ii) a promoter; and c) introducing the vector into a host cell under time and conditions sufficient for expression of the elongase enzyme. The properties of the host cell are the same as those described above in connection with SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3.

The present invention includes also encompasses a vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:4 (Figure 46) operably linked to b) a promoter, as well as a host cell comprising this vector. The host cell has the same properties as those recited above in connection with the host cell recited above for SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

Moreover, the present invention includes a plant cell, plant or plant tissue comprising the above vector comprising SEQ ID NO:4, wherein expression of said nucleotide sequence of the vector results in production of a polyunsaturated fatty acid by

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the plant cell, plant or plant tissue. The polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA. The invention also includes one or more plant oils or fatty acids expressed by this plant cell, plant or plant tissue.

The invention also includes transgenic plant comprising the above vector including the nucleotide sequence corresponding to SEQ ID NO:4, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in seeds of the transgenic plant.

Additionally, the present invention includes a transgenic, non-human mammal whose genome comprises a *C. elegans* DNA sequence encoding an elongase operably linked to a promoter. The DNA sequence may be represented by SEQ ID NO:4 (Figure 46). The invention also includes a fluid produced by the transgenic, non-human mammal, wherein the fluid comprises a detectable level of at least one elongase or products thereof.

The invention also includes a method for producing a polyunsaturated fatty acid comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:4 (Figure 46); b) constructing a vector comprising the isolated nucleotide sequence; c) introducing the vector into a host cell under time and conditions sufficient for expression of an elongase enzyme encoded by the isolated nucleotide sequence; and d) exposing the expressed elongase enzyme to a substrate polyunsaturated fatty acid in order to convert the substrate to a product polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be, for example, GLA, STA, or AA, and the product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA, respectively. The method may further comprise the step of exposing the expressed elongase enzyme to at least one desaturase in order to convert said product polyunsaturated

fatty acid to another polyunsaturated fatty acid. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3 or ADA, the another polyunsaturated fatty acid may be, for example, AA, EPA or  $\omega$ 6-docosapentaenoic acid, respectively, and the at least one desaturase is  $\Delta$ 5-desaturase with respect to production of AA or EPA, and  $\Delta$ 4-desaturase with respect to production of  $\omega$ 6-docosapentaenoic acid. The method may further comprise the step of exposing the another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert the another polyunsaturated fatty acid to a final polyunsaturated fatty acid. The final polyunsaturated fatty acid may be, for example, DHA, ADA,  $\omega$ 6-docosapentaenoic acid, or  $\omega$ 3-docosapentaenoic acid.

The invention also includes a nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said the polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:4, the another polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:4, and the final polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA, or  $\omega$ 6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, ADA,  $\omega$ 6-docosapentaenoic acid, or  $\omega$ 3-docosapentaenoic acid. The other characteristics of the composition are the same as those recited for the nutritional compositions present above.

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Additionally, the present invention includes a pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4, the another polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4, and the final polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4, the pharmaceutical acceptable carrier. The composition has the same properties (e.g., administration, added elements, etc.) as those described above with respect to the other pharmaceutical compositions.

The present invention also includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:4, the another polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4, and the final polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:4. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3 or ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA or  $\omega$ 6-docosapentaenoic acid. The polyunsaturated fatty acid may be, for example, DHA, ADA,  $\omega$ 6-docosapentaenoic acid or  $\omega$ 3-docosapentaenoic acid.

Additionally, the present invention includes a cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced

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according to the method recited above in connection with SEQ ID NO:4, the another polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4 and the final polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:4.

Furthermore, the present invention encompasses a method of preventing or treating a condition caused by insufficient intake or production of polyunsaturated fatty acids comprising administering to the patient the nutritional composition recited 10 with respect to SEQ ID NO:4 in an amount sufficient to effect the treatment or prevention.

The present invention also includes an isolated nucleotide sequence corresponding to or complementary to at least about 35<sup>t</sup> of the nucleotide sequence comprising SEQ ID NO:5 (Figure 54). Thus, the sequence may be that represented by SEQ ID NO:5. The sequence may encode a functionally active elongase which utilizes a polyunsaturated fatty acid as a substrate. It may also be derived from a mammal such as, for example, a mouse. The present invention also includes a purified protein encoded 15 by the nucleotide sequence as well as a purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of the protein.

Additionally, the invention also includes a method of 20 producing an elongase enzyme, as described above, in which the nucleotide sequence isolated comprises either SEQ ID NO:5 or SEQ ID NO:6. The host cell utilized may be as described above. The present invention also encompasses a vector comprising:

a) a nucleotide sequence comprising SEQ ID NO:5 (Figure 54) (or a 25 nucleotide sequence comprising SEQ ID NO:6 (Figure 58)) operably linked to b) a promoter, as well as a host cell comprising this

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vector. Again, the host cell may be as described above for the related methods using the other nucleotide sequences of the present invention.

Additionally, the invention includes a plant cell, plant or plant tissue comprising the vector comprising SEQ ID NO:5 or 6, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid by the plant cell, plant or plant tissue. When the nucleotide sequence of the vector comprises SEQ ID NO:5, the polyunsaturated fatty acid is selected from the group consisting of AA, ADA, GLA and STA. The invention also includes one or more plant oils or acids expressed by the plant cell, plant or plant tissue.

The present invention also includes a transgenic plant comprising the vector described above, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in seeds of the transgenic plant. Additionally, the present invention encompasses a transgenic, non-human mammal whose genome comprises a DNA sequence encoding an elongase, operably linked to a promoter, wherein the DNA sequence comprises SEQ ID NO:5 (Figure 54) (or SEQ ID NO:6 (Figure 58)). Also, the invention includes a fluid produced by this transgenic, non-human mammal, wherein the fluid comprises a detectable level of at least one elongase or products thereof.

The invention also includes a method for producing a polyunsaturated fatty acid, similar to the methods described above, except that the isolated nucleotide sequence comprises SEQ ID NO:5 (Figure 54). The substrate polyunsaturated fatty acid may be selected from the group consisting of GLA, STA, AA, ADA and ALA, and the product polyunsaturated fatty acid may be

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selected from the group consisting of DGLA, 20:4n-3, ADA,  $\omega$ 6-docosapentaenoic acid and STA, respectively. The method may further comprise the step of exposing the expressed elongase enzyme to at least one desaturase in order to convert the product polyunsaturated fatty acid to another polyunsaturated fatty acid. The product polyunsaturated fatty acid may be selected from the group consisting of DGLA, 20:4n-3, ADA and  $\omega$ 6-docosapentaenoic acid, the another polyunsaturated fatty acid is selected from the group consisting of AA, EPA,  $\omega$ 6-docosapentaenoic acid and docosahexaenoic acid respectively, and the at least one desaturase is  $\Delta$ 5-desaturase with respect to the production of AA or EPA, and  $\Delta$ 4-desaturase with respect to production of  $\omega$ 6-docosapentaenoic acid, and  $\Delta$ 19-desaturase with respect to production of docosahexaenoic acid. The method may further comprises the step of exposing the another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert the another polyunsaturated fatty acid to a final polyunsaturated fatty acid. The final polyunsaturated fatty acid may selected from the group consisting of ADA,  $\omega$ 3-docosapentaenoic acid and docosahexaenoic acid.

The present invention also includes a nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the method above, the another polyunsaturated fatty acid is produced according to the method above, and the final polyunsaturated fatty acid produced according to the method above. The product polyunsaturated fatty acid may be selected from the group

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consisting of DGLA, 20:4n-3, ADA, and  $\omega$ 6-docosapentaenoic acid and STA. The another polyunsaturated fatty acid is selected from the group consisting of AA, EPA,  $\omega$ 6-docosapentaenoic acid and docosahexaenoic acid. The final polyunsaturated fatty acid is selected from the group consisting of ADA,  $\omega$ 3-docosapentaenoic acid and docosahexaenoic acid. The nutritional composition may be selected from the group consisting of an infant formula, a dietary supplement and a dietary substitute.

The present invention also includes a pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the method above, the another polyunsaturated fatty acid produced according to the method above, and the final polyunsaturated fatty acid produced according to the method above and 2) a pharmaceutically acceptable carrier.

Additionally, the present invention includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the method above, the another polyunsaturated fatty acid produced according to the method above and the final polyunsaturated fatty acid produced according to the method above. The product polyunsaturated fatty acid may be selected from the group consisting of DGLA, 20:4n-3, ADA,  $\omega$ 6-docosapentaenoic acid and STA. The another polyunsaturated fatty acid may be selected from the group consisting of AA, EPA,  $\omega$ 6-docosapentaenoic acid and docosahexaenoic acid. The final polyunsaturated fatty acid may be selected from the group consisting of ADA,  $\omega$ 3-docosapentaenoic acid and docosahexaenoic acid.

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The invention includes a cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the method above, the another polyunsaturated fatty acid produced according to the method above and the final polyunsaturated fatty acid produced according to the method above.

Additionally, a method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to the patient the nutritional composition above in an amount sufficient to effect the prevention or treatment.

The present invention includes an isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence comprising SEQ ID NO:6 (Figure 5B).

15 The isolated nucleotide sequence may comprise SEQ ID NO:6. The invention also includes a purified protein encoded by the nucleotide sequences.

Additionally, the present invention relates to an isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence shown in SEQ ID NO:7 (Figure 72). This isolated sequence may be represented by SEQ ID NO:7. The sequence encodes a functionally active elongase which utilizes a polyunsaturated fatty acid or a monounsaturated fatty acid as a substrate. In particular, the sequence may be 20 derived from a fungus of the genus Thraustochytrium aureum and may specifically be isolated from Thraustochytrium aureum.

The present invention also includes a purified protein encoded by the above nucleotide sequence as well as a purified polypeptide which elongates polyunsaturated fatty acids or 25 monounsaturated fatty acids and has at least about 50 amino

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acid similarity to the amino acid sequence of the purified protein encoded by the above nucleotide sequence.

Additionally, the present invention encompasses a method of producing an elongase enzyme comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:7 (Figure 72); b) constructing a vector comprising: i) the isolated nucleotide sequence operably linked to ii) a promoter; and c) introducing the vector into a host cell under time and conditions sufficient for expression of the elongase enzyme. The host cell may be a eukaryotic cell or a prokaryotic cell. The prokaryotic cell may be, for example an E. coli cell, a cyanobacterial cell, or a B. subtilis cell. The eukaryotic cell may be, for example, a mammalian cell, an insect cell, a plant cell or a fungal cell. The fungal cell may be, for example, Saccharomyces spp., Candida spp., Lipomyces spp., Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. or Pichia spp.. In particular, the fungal cell may be a yeast cell such as Saccharomyces spp., in particular, Saccharomyces cerevisiae, Candida spp., Hansenula spp. or Pichia spp.. The invention also includes a vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:7 (Figure 72) operably linked to b) a promoter, as well as a host cell comprising this vector. The host may be a prokaryotic cell or a eukaryotic cell. Suitable examples of prokaryotic cells include E. coli, Cyanobacteria, and B. subtilis cells. Suitable examples of eukaryotic cells include a mammalian cell, an insect cell, a plant cell and a fungal cell. The fungal cell may be, for example, Saccharomyces spp., Candida spp., Lipomyces spp., Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. and Trichodema spp.

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Pichia spp. In particular, the fungal cell may be, for example, a yeast cell such as, for example, Saccharomyces spp., in particular, Saccharomyces cerevisiae, Candida spp., Hansenula spp. and Pichia spp..

5 The present invention includes a plant cell, plant or plant tissue comprising the above-described vector, wherein expression of the nucleotide sequence of the vector results in production of at least one fatty acid selected from the group consisting of a monounsaturated fatty acid and a polyunsaturated fatty acid by 10 the plant cell, plant or plant tissue. The polyunsaturated fatty acid may be, for example, dihomo- $\gamma$ -linolenic acid (DGLA), 20:4n-3, and adrenic acid (ADA). The invention also includes one or more plant oils or fatty acids expressed by the plant cell, plant or plant tissue. Additionally, the present invention encompasses a transgenic plant comprising the above-described vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in seeds of the transgenic plant.

15 Furthermore, the present invention includes a transgenic, non-human mammal whose genome comprises a DNA sequence encoding an elongase operably linked to a promoter. The DNA sequence may be represented by SEQ ID NO:7 (Figure 72). The present invention also includes a fluid (e.g., milk) produced by the transgenic, non-human wherein the fluid comprises a detectable 20 level of at least one elongase or products thereof such as, for example, DGLA,  $\omega$ 6-docosapentaenoic acid, ADA and/or 20:4n-3 (see Figure 1).

25 Additionally, the present invention includes a method for producing a polyunsaturated fatty acid comprising the steps of:

30 a) isolating said nucleotide sequence represented by SEQ ID NO:7

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(Figure 72); b) constructing a vector comprising the isolated nucleotide sequence; c) introducing the vector into a host cell under time and conditions sufficient for expression of elongase enzyme encoded by the isolated nucleotide sequence; and d) exposing the expressed elongase enzyme to a "substrate" polyunsaturated fatty acid in order to convert the substrate to a "product" polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be selected from the group consisting of, for example,  $\gamma$ -linolenic acid (GLA), stearidonic acid (STA) and arachidonic acid (AA), and the product polyunsaturated fatty acid may be selected from the group consisting of, for example, DGLA, 20:4n-3, and ADA, respectively. The method may further comprise the step of exposing the product polyunsaturated fatty acid to at least one desaturase in order to convert the product polyunsaturated fatty acid to "another" polyunsaturated fatty acid. The product polyunsaturated fatty acid may be selected from the group consisting of, for example, DGLA, 20:4n-3, and ADA. The another polyunsaturated fatty acid may be selected from the group consisting of, for example, AA, eicosapentaenoic acid (EPA),  $\omega$ 6-docosapentaenoic acid, respectively, and the at least one desaturase is  $\Delta$ 5-desaturase, with respect to production of AA or EPA, and  $\Delta$ 4-desaturase, with respect to production of  $\omega$ 6-docosapentaenoic acid. The method may further comprise the step of exposing the another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert the another polyunsaturated fatty acid to a "final" polyunsaturated fatty acid. The final polyunsaturated fatty acid may be, for example, docosahexaenoic acid (DHA), AA,  $\omega$ 6-docosapentaenoic acid, or  $\omega$ 3-docosapentaenoic acid.

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Also, the present invention includes a nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another polyunsaturated fatty acid produced according to the above-described method, and the final polyunsaturated fatty acid produced according to the above-described method. The product polyunsaturated fatty acid may be selected from the group consisting of, for example, DGLA, 20:4n-3 and ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA, or  $\omega$ 6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, adrenic acid,  $\omega$ 6-docosapentaenoic acid or  $\omega$ 3-docosapentaenoic acid. The nutritional composition may be, for example, an infant formula, a dietary supplement or a dietary substitute and may be administered to a human or an animal and may be administered enterally or parenterally. The nutritional composition may further comprise at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, monoglycerides, diglycerides, glucoses, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, protein hydrolysates, sunflower oil, safflower oil, corn oil, and flax oil. It may also comprise at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex and at least one mineral selected from the group consisting of calcium magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium and iron.

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Additionally, the present invention encompasses a pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another product polyunsaturated fatty acid produced according to the above-described method, the another polyunsaturated fatty acid produced according to the above-described method, and the final polyunsaturated fatty acid produced according to the above-described method.

10 The composition may be administered to a human or an animal. It may also further comprise at least one element selected from the group consisting of a vitamin, a mineral, a salt, a carbohydrate, an amino acid, a free fatty acid, a preservative, an excipient, an anti-histamine, a growth factor, an antibiotic, a diluent, a phospholipid, an antioxidant, and a phenolic compound. It may be administered enterally, parenterally, topically, rectally, intramuscularly, subcutaneously, intradermally, or by any other appropriate means.

15 The present invention also includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another polyunsaturated fatty acid produced according to the above-described method, and the final polyunsaturated fatty acid produced according to the above-described method. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n3, and ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA, or  $\omega$ 3-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, adrenic acid,  $\omega$ 6-docosapentaenoic acid or  $\omega$ 3-docosapentaenoic acid.

20 Moreover, the present invention also includes a cosmetic

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comprising a polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another polyunsaturated fatty acid produced according to the above-described method, and the final polyunsaturated fatty acid produced according to the above-described method.

5 Additionally, the present invention includes a method of preventing or treating a condition caused by insufficient intake or production of polyunsaturated fatty acids comprising administering to the patient the above nutritional composition in an amount sufficient to effect prevention or treatment.

10 All U.S. patents and publications referred to herein are hereby incorporated in their entirety by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 represents various fatty acid biosynthesis pathways. The role of the elongase enzyme (elo) should be noted.

15 Figure 2 represents the percent similarity and percent identity between the amino acid sequences of jojoba KCS and ELO2.

20 Figure 3 represents the S. cerevisiae ELO2 sequence homologous to the jojoba KCS sequence (primer sequence underlined) of Figure 2.

25 Figure 4A shows the physical map of PRAE-2 containing the MABLO cDNA. Figure 4B represents the physical map of the constitutive expression vector, PRAE-5, used for elongase enzyme production in yeast.

30 Figure 5 represents a comparison of the nucleotide sequences of clones PRAE-5 and PRAE-6.

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Figure 6 illustrates the complete nucleotide sequence of Mortierella alpina elongase (MAELO).

Figure 7 represents the amino acid sequence of the Mortierella alpina elongase translated from MAELO (see Figure 6).

Figure 8 represents an amino acid sequence alignment among 3 elongases: S. cerevisiae ELO2 (GNS1), S. cerevisiae ELO3 (STR4) and the translated MAELO sequence as shown in Figure 7.

Figure 9 represents a comparison between the nucleotide 10 sequence MAELO and the nucleotide sequence of ELO2 from S. cerevisiae.

Figures 10A and 10B represents the PUFA elongase activity of MAELO expressed in baker's yeast.

Figure 11 illustrates the PUFA elongase activity of MAELO 15 when co-expressed with the  $\Delta 5$ -desaturase cDNA from M. alpina to produce AA.

Figure 12 compares the PUFA elongase activity of MAELO to the overexpression of ELO2 from S. cerevisiae in baker's yeast.

Figures 13, 14 and 15 represent three separate comparisons 20 of amino acid sequences derived from C. elegans nucleotide sequences in the GenEMBL database with the translated MAELO.

Figure 16 shows the comparison between amino acid translations of two different mammalian sequences in the GenEMBL database and the translated MAELO.

Figure 17 shows the comparison of a translated DNA sequence 25 (see published PCT application WO 98/07577) with the amino acid sequence derived from MAELO, which was detected during a database search.

Figure 18 shows the complete nucleotide sequence of the  $\Delta 5$ - 30 desaturase from M. alpina.

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Figure 19 represents the initial GC-FAME analysis of MAD708 pool. The detection of a DGLA (C20:3n-6) peak should be noted.

Figure 20 represents the PUFA elongase activity of the five MAD708 clones in yeast with GLA as substrate. All clones have 5 apparent elongase activity.

Figure 21 represents the DNA sequencing analysis of plasmid pPRPB2. The analysis reveals an open reading frame of 957 bp in pPRPB2.

Figure 22 shows the complete nucleotide sequence of the M. alpina cDNA, contained in the plasmid pPRPB2, which is designated GLELO for its GLA elongase activity.

Figure 23 represents the amino acid sequence of the M. alpina elongase translated from GLELO (see Figure 22).

Figure 24 illustrates the n-6 PUFA elongase activity in an 15 induced culture of 334 (pPRPB2) when supplemented with GLA.

Figure 25 represents the n-3 and n-6 PUFA elongase activity in an induced culture of 334 (pPRPB2) when supplemented with 25  $\mu$ M of other fatty acid substrates.

Figure 26A illustrates the elongase activity of GLELO with 20 GLA as a substrate when co-expressed with the M. alpina  $\Delta 5$ -desaturase cDNA to produce AA. Figure 26B illustrates the elongase activity of GLELO with STA as a substrate when co-expressed with the M. alpina  $\Delta 5$ -desaturase cDNA to produce EPA.

Figure 27 illustrates the comparison between the translated 25 GLELO sequence (see Figure 23) and the translated MAELO sequence (see Figure 7).

Figure 28 represents a comparison of the amino acid sequence of 4 elongases: the translated amino acid sequence of GLELO (see Figure 23), MAELO (see Figure 7), S. cerevisiae ELO2

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(HS1), and *S. cerevisiae* ELO3 (SUR4). The histidine box is underlined.

Figure 29 represents an alignment between translated MAELO sequence and translated putative human homologue HS1 sequence.

Figure 30 represents an alignment between the translated MAELO sequence and the translated putative human homologue HS2 sequence.

Figure 31 shows an alignment between the translated MAELO sequence and the translated putative mouse homologue MM2 sequence.

Figure 32 represents an alignment between the translated MAELO and the translated putative mouse homologue AI225632 sequence.

Figure 33 illustrates an alignment between the translated MAELO sequence and the translated human homologue AI815960 sequence.

Figure 34 shows an alignment between the translated GLELO sequence and the translated putative human homologue HS1 sequence.

Figure 35 represents an alignment between the translated GLELO sequence and the translated putative human homologue sequence from AC004050.

Figure 36 illustrates an alignment between the translated GLELO sequence and the translated putative mouse homologue MM2 sequence.

Figure 37 represents an alignment of the translated GLELO sequence and a translated putative mouse homologue AI225632 sequence.

Figure 38 illustrates an alignment of the translated GLELO sequence and a translated putative mouse homologue U97107.

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Figure 39 represents an alignment of the translated GLELO sequence and a translated putative *C. elegans* U68749 (F56H11.4) homologue sequence.

Figure 40 shows an alignment between the translated MAELO sequence and a translated putative *C. elegans* U68749 (F56H11.4) homologue sequence.

Figure 41 represents an alignment between the translated GLELO sequence and a translated putative *Drosophila melanogaster* homologue sequence, DM1.

Figure 42 illustrates an alignment between the translated MAELO sequence and a translated putative *Drosophila melanogaster* homologue sequence, DM1.

Figure 43 illustrates the complete nucleotide sequence of a human elongase HSEL01.

Figure 44 represents the deduced amino acid sequence of the human elongase HSEL01.

Figure 45 illustrates the elongase activity (PUFA and others) of an induced culture of 334 (pRAE-58-A1) when supplemented with GLA or AA.

Figure 46 shows the complete nucleotide sequence of the *C. elegans* elongase CEELO.

Figure 47 represents the deduced amino acid of *C. elegans* elongase CEELO.

Figure 48 illustrates the PUFA elongase activity of an induced culture of 334 (pRET-21) and 334 (pRET-22) when supplemented with GLA and AA.

Figure 49 represents the complete nucleotide sequence of the putative human elongase gene HS3.

Figure 50 illustrates the deduced amino acid sequence of the putative human elongase enzyme HS3.

Figure 51 represents the elongase activity (PUFA and others) of HSEL01 expressed in baker's yeast when supplemented with GLA, AA, STA, EPA, OA, or ALA.

Figure 52 represents the elongase activity (PUFA and others) of HSEL01 expressed in baker's yeast when supplemented with 25 mM or 100 mM of GLA, AA, or EPA.

Figures 53A, 53B, and 53C represent the elongase activity (PUFA and others) of HSEL01 expressed in baker's yeast when supplemented with PA, SA, ARA, BA, PTA, OA, EA, LA, GLA, DGLA, AA, ADA, ALA, STA, EPA, or DPA, or when no substrate is present.

Figure 54 represents the complete nucleotide sequence of mouse elongase MELO4.

Figure 55 represents the deduced amino acid sequence of the mouse elongase MELO4.

Figure 56 represents the PUFA elongase activity of MELO4 expressed in baker's yeast when supplemented with GLA, AA, ADA, STA, EPA, or DPA.

Figures 57A, 57B, and 57C represent the PUFA elongase activity of MELO4 expressed in baker's yeast when supplemented with PA, SA, ARA, BA, PTA, OA, EA, LA, GLA, DGLA, AA, ADA, STA, EPA, or DPA, or when no substrate is present.

Figure 58 represents the complete nucleotide sequence of mouse elongase MELO7.

Figure 59 represents the deduced amino acid sequence of the mouse elongase MELO7.

Figure 60 represents the elongase activity (PUFA and others) of MELO7 expressed in baker's yeast when supplemented with GLA, AA, ADA, STA, EPA, or DPA.

Figure 61 shows the activity of the *C. elegans* elongase when expressed in yeast when no substrate is present and with addition of AA or GLA.

Figure 62 illustrates the PUFA elongase activity of an induced culture of 334 (pRET22) when supplemented with 50  $\mu$ M of various substrates.

Figure 63 represents the PUFA elongase activity with GLA (Figure 63A) or STA (Figure 63B) as a substrate when co-expressed with the *M. alpina*  $\Delta 5$ -desaturase cDNA to produce AA or EPA, respectively.

Figure 64 represents an amino acid sequence alignment among 4 elongases: HSEL01 (Figure 44), MELO4 (Figure 55), GLELO 10 (Figure 23), and CEELO (Figure 47).

Figure 65 represents the consensus nucleotide sequence of the partial *Thraustochytrium aureum* (*T. aureum*) 7091 elongase gene.

Figure 66 represents the deduced amino acid sequence of the *T. aureum* 7091 elongase gene in Figure 65.

Figure 67 represents the complete nucleotide sequence of the *T. aureum* 7091 elongase TELO1, from plasmid PRAT-4-A1.

Figure 68 represents the complete nucleotide sequence of the *T. aureum* 7091 elongase TELO1, from plasmid PRAT-4-A2.

Figure 69 represents the complete nucleotide sequence of the *T. aureum* 7091 elongase TELO1, from plasmid PRAT-4-A3.

Figure 70 represents the complete nucleotide sequence of the *T. aureum* 7091 elongase TELO1, from plasmid PRAT-4-A4.

Figure 71 represents the complete nucleotide sequence of the *T. aureum* 7091 elongase TELO1, from plasmid PRAT-4-A6.

Figure 72 represents the complete nucleotide sequence of the *T. aureum* 7091 elongase TELO1, from plasmid PRAT-4-A7.

Figure 73 represents the complete nucleotide sequence of the *T. aureum* 7091 elongase TELO1, from plasmid PRAT-4-D1.

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Figure 74 represents the deduced amino acid sequence of the *T. aureum* 7091 elongase TELO1, from plasmid PRAT-4-A1.

Figure 75 represents the deduced amino acid sequence of the *T. aureum* 7091 elongase TELO1, from plasmid PRAT-4-A2.

Figure 76 represents the deduced amino acid sequence of the *T. aureum* 7091 elongase TELO1, from plasmid PRAT-4-A3.

Figure 77 represents the deduced amino acid sequence of the *T. aureum* 7091 elongase TELO1, from plasmid PRAT-4-A4.

Figure 78 represents the deduced amino acid sequence of the *T. aureum* 7091 elongase TELO1, from plasmid PRAT-4-A6.

Figure 79 represents the deduced amino acid sequence of the *T. aureum* 7091 elongase TELO1, from plasmid PRAT-4-A7.

Figure 80 represents the deduced amino acid sequence of the *T. aureum* 7091 elongase TELO1, from plasmid PRAT-4-D1.

Figure 81 represents the elongase activity of TELO1 expressed in baker's yeast when supplemented with GLA or EPA.

Figure 82 represents an amino acid sequence alignment among the TELO1 elongases from the 7 plasmids: PRAT-4-A1 (Figure 74), PRAT-4-A2 (Figure 75), PRAT-4-A3 (Figure 76), PRAT-4-A4 (Figure 77), PRAT-4-A6 (Figure 78), PRAT-4-A7 (Figure 79), and PRAT-4-D1 (Figure 80).

Figure 83 represents an alignment between the translated TELO1 sequence and the translated HSELO1 sequence.

Figure 84 represents an alignment between the translated TELO1 sequence and the translated MELO4 sequence.

Figure 85 represents an alignment between the translated TELO1 sequence and the translated GLELO sequence.

Figure 86 represents an alignment between the translated TELO1 sequence and the translated CBELO sequence.

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Figure 87 represents the elongase activity of TELO1 expressed in baker's yeast when supplemented with GLA, AA, STA, EPA, or no substrate.

Figure 88 represents the elongase activity of TELO1 expressed in baker's yeast when supplemented with LA, ALA, GLA, STA, DGLA, ETA, AA or EPA.

#### DETAILED DESCRIPTION OF THE INVENTION

The subject invention relates to nucleotide and corresponding amino acid sequences of two elongase cDNAs derived from *Mortierella alpina*, as well as to nucleotide and corresponding amino acid sequences of an elongase cDNA derived from a human, an elongase cDNA derived from *C. elegans*, two elongase cDNAs derived from a mouse, and an elongase cDNA derived from *Thraustochytrium aureum*. Furthermore, the subject invention also includes uses of the cDNAs and of the proteins encoded by the genes. For example, the genes and corresponding enzymes may be used in the production of polyunsaturated fatty acids and/or monounsaturated fatty acids such as, for example, DGLA, AA, ADA, EPA and/or DHA which may be added to pharmaceutical compositions, nutritional compositions, animal feeds, cosmetics, and to other valuable products.

#### 25 The Elongase Genes and Enzymes Encoded Thereby

As noted above, an elongase enzyme encoded by an elongase cDNA is essential in the production of various polyunsaturated fatty acids, in particular, 20-24 carbon PUFAs. With respect to the present invention, the nucleotide sequence of the isolated *M. alpina* elongase cDNA (MABLO) is shown in Figure 6, and the amino acid sequence of the corresponding purified protein or

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Figure 87 represents an alignment between the translated TELO1 sequence and the translated GLELO sequence.

Figure 88 represents an alignment between the translated TELO1 sequence and the translated CBELO sequence.

Figure 89 represents an alignment between the translated TELO1 sequence and the translated HSELO sequence.

Figure 90 represents an alignment between the translated TELO1 sequence and the translated MELO4 sequence.

Figure 91 represents an alignment between the translated TELO1 sequence and the translated CBELO sequence.

Figure 92 represents an alignment between the translated TELO1 sequence and the translated HSELO sequence.

Figure 93 represents an alignment between the translated TELO1 sequence and the translated GLELO sequence.

Figure 94 represents an alignment between the translated TELO1 sequence and the translated CBELO sequence.

enzyme encoded by this nucleotide sequence is shown in Figure 7. Additionally, the nucleotide sequence of the isolated GLA elongase cDNA (GLELO) is shown in Figure 22, and the amino acid sequence of the corresponding purified protein or enzyme encoded by this nucleotide sequence is shown in Figure 23. The nucleotide sequence of the isolated human sequence 1 (HSEL01) elongase is shown in Figure 43, and the amino acid sequence of the corresponding purified protein or enzyme encoded by this sequence is shown in Figure 44. Furthermore, the nucleotide sequence of the isolated *C. elegans* elongase cDNA (CEELO1) is shown in Figure 46, and the amino acid sequence of the corresponding purified protein or enzyme encoded thereby is shown in Figure 47. Additionally, the nucleotide sequence of the isolated mouse PUFA elongation enzyme (MELO4) is shown in Figure 54, and the amino acid sequence of the corresponding purified protein or enzyme encoded thereby is shown in Figure 55. Moreover, the nucleotide sequence of the second isolated mouse PUFA elongation enzyme (MELO7) is shown in Figure 58, and the amino acid sequence of the corresponding purified protein or enzyme encoded thereby is shown in Figure 59. Also, the nucleotide sequence of the isolated *T. aureum* elongase cDNA (TELO1) is shown in Figure 72, and the amino acid sequence of the corresponding purified protein of enzyme encoded thereby is shown in Figure 79.

As an example, several of the isolated elongases encoded by the cDNAs of the present invention elongate GLA to DGLA or elongate STA to 20:4n-3 or elongate AA to ADA. The production of arachidonic acid from DGLA, or EPA from 20:4n-3, is then catalyzed by, for example, a Δ5-desaturase. Thus, neither AA (or EPA), nor DGLA (or 20:4n-3) nor ADA (or ω3-docosapentaenoic

acid), can be synthesized without at least one elongase cDNA and enzyme encoded thereby.

It should be noted that the present invention also encompasses nucleotide sequences (and the corresponding encoded proteins) having sequences corresponding to (i.e., having identity to) or complementary to at least about 50% of the nucleotides in SEQ ID NO:1 (i.e., the nucleotide sequence of the MEL0 cDNA described herein (see Figure 6)). Furthermore, the present invention also includes nucleotide sequences (and the corresponding encoded proteins) having sequences corresponding to (i.e., having identity to) or complementary to at least about 35%, preferably at least about 55% of the nucleotides in SEQ ID NO:2 (i.e., the nucleotide sequence of the GLELO cDNA described herein (see Figure 22)). Additionally, the present invention also includes nucleotide sequences (and the corresponding encoded proteins) having sequences corresponding to (i.e., having identity to) or complementary to at least about 35%, preferably at least about 45%, and more preferably at least about 55% of the nucleotides in SEQ ID NO:3 (i.e., the nucleotide sequence of the human sequence 1 (HSEL01) cDNA described herein (see Figure 43)). In addition, the present invention also includes nucleotide sequences (and the corresponding encoded proteins) having sequences corresponding to (i.e., having identity to) or complementary to at least about 35%, preferably at least about 45%, and more preferably at least about 55% of the nucleotides in SEQ ID NO:4 (i.e., the nucleotide sequence of the *C. elegans* cDNA, CEELO1, described herein (see Figure 46)). Further, the present invention also includes nucleotide sequences (and the corresponding encoded

proteins) having sequences corresponding to (i.e., having identity to) or complementary to at least about 35%, preferably at least about 45%, and more preferably at least about 55% of the nucleotides in SEQ ID NO:5 or SEQ ID NO:6 (i.e., the nucleotide sequence of the mouse PUFA elongases MELO4 and MELO7, described herein (see Figures 54 and 58, respectively)). Also, the present invention encompasses nucleotide sequences (and the corresponding encoded proteins) having sequences corresponding to (i.e., having identity to) or complementary to at least about 35%, preferably at least about 45%, and more preferably at least about 55% of the nucleotides in SEQ ID NO:7 (i.e., the nucleotide sequence of the *T. aureum* elongase gene TELO1, described herein (see Figure 72)). It should be noted that the "most preferable" range, referred to in each instance, may be increased by increments of ten percent. For example, if "at least 55%" is the most preferable range recited above, with respect to a particular sequence, such a range also naturally includes "at least 65% identity", "at least 75% identity", "at least 85% identity", and "at least 95% identity".

The corresponding or complementary sequences may be derived from non-*Mortierella* sources or sources other than from which the isolated, original sequences were derived (e.g., a eukaryote (e.g., *Thraustochytrium* spp. (e.g., *Thraustochytrium aureum* and *Thraustochytrium roseum*), *Schizochytrium* spp. (e.g., *Schizochytrium aggregatum*), *Conidiobolus* spp. (e.g., *Conidiobolus nanodes*), *Entomophthora* spp. (e.g., *Entomophthora exitalis*), *Saprolegnia* spp. (e.g., *Saprolegnia parasitica* and *Saprolegnia diclina*), *Leptomitus* spp. (e.g., *Leptomitus lacteus*), *Entomophthora* spp., *Pythium* spp., *Porphyridium* spp. (e.g., *Porphyridium cruentum*), *Conidiotholus* spp., *Phytophthora* spp., *Penicillium* spp., *Coidosporium* spp., *Mucor* spp. (e.g.,

5 *Mucor circinelloides* and *Mucor javanicus*), *Fusarium* spp., *Aspergillus* spp., *Rhodotorula* spp., *Amphidinium carteri*, *Chaetoceros calcitrans*, *Cricosphaera carterae*, *Cryptocodinium cohnii*, *Cryptomonas ovata*, *Euglena gracilis*, *Gonyaulax polyedra*, *Gymnodinium* spp. (e.g. *Gymnodinium nelsoni*), *Gyrodinium cohnii*, *Isochrysis* spp. (e.g. *Isochrysis galbana*), *Microalgae* MK8805,

10 *Nitzschia frustulum*, *Pavlova* spp. (e.g. *Pavlova lutheri*), *Phaeodactylum tricornutum*, *Prorocentrum* spp., *Rhodomonas* spp., and *Thalassiosira pseudonana*), a Psychrophilic bacteria (e.g., *Vibrio* spp. (e.g., *Vibrio marinus*)), a yeast (e.g., *Dipodascopis uninucleata*), a non-mammalian organism such as a fly (e.g., *Drosophila melanogaster*) or *Caenorhabditis* spp. (e.g., *Caenorhabditis elegans*), or a mammal (e.g., a human or a mouse). Such sequences may also be derived from a species 15 within the genus *Mortierella*, other than the species *alpina*, for example, *Mortierella elongata*, *Mortierella exigua*, *Mortierella isabellina*, *Mortierella hygrophila*, and *Mortierella ramanniana*, *va. angulispora*.

Furthermore, the present invention also encompasses

20 fragments and derivatives of the nucleotide sequences of the Present invention (i.e., SEQ ID NO:1 (MABLO), SEQ ID NO:2 (GLEO), SEQ ID NO:3 (HSELO), SEQ ID NO:4 (CEELO1), SEQ ID NO:5 (MELO), SEQ ID NO:6 (MEL07) and SEQ ID NO:7 (TELO1)) as well as of the corresponding sequences derived from non- 25 *Mortierella* or non-mammalian sources, etc., as described above, and having the above-described complementarity or correspondence/identity to the 7 sequences. Functional equivalents of the above-sequences (i.e., sequences having elongase activity) are also encompassed by the present 30 invention.

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For purposes of the present invention, "complementarity" is defined as the degree of relatedness between two DNA segments. It is determined by measuring the ability of the sense strand of one DNA segment to hybridize with the antisense strand of the other DNA segment, under appropriate conditions, to form a double helix. In the double helix, wherever adenine appears in one strand, thymine appears in the other strand. Similarly, wherever guanine is found in one strand, cytosine is found in the other. The greater the relatedness between the nucleotide sequences of two DNA segments, the greater the ability to form hybrid duplexes between the strands of two DNA segments.

"Identity" between two nucleotide sequences is defined as the degree of sameness, correspondence or equivalence between the same strands (either sense or antisense) of two DNA segments. The greater the percent identity, the higher the correspondence, sameness or equivalence between the strands.

"Similarity" between two amino acid sequences is defined as the presence of a series of identical as well as conserved amino acid residues in both sequences. The higher the degree of similarity between two amino acid sequences, the higher the correspondence, sameness or equivalence of the two sequences. ("Identity" between two amino acid sequences is defined as the presence of a series of exactly alike or invariant amino acid residues in both sequences.)

25 The definitions of "complementarity", "identity", and "similarity" are well known to those of ordinary skill in the art.

The invention also includes a purified polypeptide which elongates polyunsaturated and monounsaturated fatty acids and has at least about 50%, preferably at least about 70%, and more preferably at least about 90% amino acid similarity to the amino

acid sequences of the above-noted proteins (see, e.g., Figure 7 (MABLO) ) and which are, in turn, encoded by the above-described nucleotide sequences. Additionally, the present invention includes a purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30%, preferably at least about 60%, and more preferably at least about 90% amino acid similarity to the amino acid sequences of the above-noted proteins (see, e.g., Figure 23 (GLELO) ) and which are, in turn, encoded by the above-described nucleotide sequences.

10 Furthermore, the invention also includes a purified polypeptide which elongates polyunsaturated and monounsaturated fatty acids and has at least about 30%, preferably at least about 60%, and more preferably at least about 90% amino acid similarity to the amino acid sequences of the above-noted proteins (see, e.g.,

15 Figure 44 (HSEL01) ) and which are, in turn, encoded by the above-described nucleotide sequences. Also, the present invention includes a purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30%, preferably at least about 60%, and more preferably at least about 90% amino acid similarity to the amino acid sequences of the above-noted proteins (see, e.g.,

20 Figure 47 (CEELO1) ) and which are, in turn, encoded by the above-described nucleotide sequences. The present invention also includes a purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30%, preferably at least about 90% amino acid similarity to the amino acid sequences of the above-noted proteins (see, e.g., Figure 55 (MELO4) and Figure 58 (MEL07) ) and which are, in turn, encoded by the above-described nucleotide sequences. Also, the present

25 invention includes a purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30%,

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preferably at least about 60%, and more preferably at least about 90% amino acid similarity to the amino acid sequences of the above noted proteins (see, e.g., Figure 79 (TEL01)) and which are, in turn, encoded by the above-described nucleotide sequences.

The present invention also encompasses an isolated

nucleotide sequence which encodes PUFA elongase activity and that is hybridizable, under moderately stringent conditions, to a nucleic acid having a nucleotide sequence corresponding or complementary to the nucleotide sequence represented by SEQ ID NO:1 shown in Figure 6 (MEL01) and/or SEQ ID NO:2 shown in Figure 22 (GLE01) and/or SEQ ID NO:3 (HSELO1) shown in Figure 43 and/or SEQ ID NO:4 (CEEL01) shown in Figure 46 and/or SEQ ID NO:5 (MELO4) shown in Figure 54 and/or SEQ ID NO:6 (MELO7) shown in Figure 58 and/or SEQ ID NO:7 (TEL01) shown in Figure 72. A nucleic acid molecule is "hybridizable" to another nucleic acid molecule when a single-stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and ionic strength (see Sambrook et al., "Molecular Cloning: A Laboratory Manual, Second Edition (1989), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. "Hybridization" requires that two nucleic acids contain complementary sequences. However, depending on the stringency of the hybridization, mismatches between bases may occur. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementarity. Such variables are well known in the art.

More specifically, the greater the degree of similarity or homology between two nucleotide sequences, the greater the value

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of  $T_m$ , melting temperature, for hybrids of nucleic acids having those sequences. For hybrids of greater than 100 nucleotides in length, equations for calculating  $T_m$  have been derived (see Sambrook et al., *supra*). For hybridization with shorter nucleic acids, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook et al., *supra*).

Production of the Elongase Enzyme

Once the gene encoding the elongase has been isolated, it may then be introduced into either a prokaryotic or eukaryotic host cell through the use of a vector, plasmid or construct. The vector, for example, a bacteriophage, cosmid or plasmid, may comprise the nucleotide sequence encoding the elongase as well as any promoter which is functional in the host cell and is able to elicit expression of the elongase encoded by the nucleotide sequence. The promoter is in operable association with or operably linked to the nucleotide sequence. (A promoter is said to be "operably linked" with a coding sequence if the promoter affects transcription or expression of the coding sequence.) Suitable promoters include, for example, those from genes encoding alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglucomomerase, phosphoglycerate kinase, acid phosphatase, T7, TPL, lactase, metallothionein, cytomegalovirus immediate early, whey acidic protein, glucoamylase, and promoters activated in the presence of galactose, for example, GAL1 and GAL10. Additionally, nucleotide sequences which encode other proteins, oligosaccharides, lipids, etc. may also be included within the vector as well as other regulatory sequences such as a polyadenylation signal (e.g., the poly-A signal of SV-40T-

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antigen, ovalbumin or bovine growth hormone). The choice of sequences present in the construct is dependent upon the desired expression products as well as the nature of the host cell. As noted above, once the vector has been constructed, it may then be introduced into the host cell of choice by methods known to those of ordinary skill in the art including, for example, transfection, transformation and electroporation (see Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., Vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press (1989)).

10 The host cell is then cultured under suitable conditions permitting expression of the PUPA which is then recovered and purified.

It should also be noted that one may design a unique triglyceride or oil if one utilizes one construct or vector comprising the nucleotide sequences of two or more cDNAs (e.g., MAELO, GIELO, HSELO1, CEELO1 and/or TELO1). This vector may then be introduced into one host cell. Alternatively, each of the sequences may be introduced into a separate vector. These vectors may then be introduced into two host cells, respectively, or into one host cell.

Examples of suitable prokaryotic host cells include, for example, bacteria such as Escherichia coli, Bacillus subtilis as well as cyanobacteria such as Spirulina spp. (i.e., blue-green algae). Examples of suitable eukaryotic host cells include, for example, mammalian cells, plant cells, yeast cells such as Saccharomyces spp., Lipomyces spp., Candida spp. such as Yarrowia (Candida) spp., Kluyveromyces spp., Pichia spp., Trichoderma spp. or Hansenula spp., or fungal cells such as filamentous fungal cells, for example, Aspergillus, Neurospora and Penicillium. Preferably, Saccharomyces cerevisiae (baker's yeast) cells are utilized.

15 Expression in a host cell can be accomplished in a transient or stable fashion. Transient expression can occur from introduced constructs which contain expression signals functional in the host cell, but which constructs do not replicate and rarely integrate in the host cell, or where the host cell is not proliferating. Transient expression also can be accomplished by inducing the activity of a regulatable promoter operably linked to the gene of interest, although such inducible systems frequently exhibit a low basal level of expression. Stable expression can be achieved by introduction of a construct that can integrate into the host genome or that autonomously replicates in the host cell. Stable expression of the gene of interest can be selected for through the use of a selectable marker located on or transfected with the expression construct, followed by selection for cells expressing the marker. When stable expression results from integration, the site of the construct's integration can occur randomly within the host genome or can be targeted through the use of constructs containing regions of homology with the host genome sufficient to target recombination with the host locus. Where constructs are targeted to an endogenous locus, all or some of the transcriptional and translational regulatory regions can be provided by the endogenous locus.

20 A transgenic mammal may also be used in order to express the enzyme of interest (i.e., the elongase) encoded by one or both of the above-described nucleotide sequences. More specifically, once the above-described construct is created, it may be inserted into the pronucleus of an embryo. The embryo may then be implanted into a recipient female. Alternatively, a nuclear transfer method could also be utilized (Schnieke et al., Science 278:2130-2133 (1997)). Gestation and birth are then

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permitted to occur (see, e.g., U.S. Patent No. 5,750,176 and U.S. Patent No. 5,700,671). Milk, tissue or other fluid samples from the offspring should then contain altered levels of PUFA<sub>s</sub>, as compared to the levels normally found in the non-transgenic animal. Subsequent generations may be monitored for production of the altered or enhanced levels of PUFA<sub>s</sub> and thus incorporation of the gene or genes encoding the elongase enzyme into their genomes. The mammal utilized as the host may be selected from the group consisting of, for example, a mouse, a rat, a rabbit, a pig, a goat, a sheep, a horse and a cow. However, any mammal may be used provided it has the ability to incorporate DNA encoding the enzyme of interest into its genome.

For expression of an elongase polypeptide, functional transcriptional and translational initiation and termination regions are operably linked to the DNA encoding the elongase polypeptide. Transcriptional and translational initiation and termination regions are derived from a variety of nonexclusive sources, including the DNA to be expressed, genes known or suspected to be capable of expression in the desired system, expression vectors, chemical synthesis, or from an endogenous locus in a host cell. Expression in a plant tissue and/or plant part presents certain efficiencies, particularly where the tissue or part is one which is harvested early, such as seed, leaves, fruits, flowers, roots, etc. Expression can be targeted to that location with the plant by utilizing specific regulatory sequence such as those of U.S. Patent Nos. 5,463,174, 4,943,674, 5,106,739, 5,175,095, 5,420,034, 5,188,958, and 5,583,379. Alternatively, the expressed protein can be an enzyme which produces a product which may be incorporated, either directly or upon further modifications, into a fluid fraction from the host plant. Expression of an elongase gene or genes, or antisense

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elongase transcripts, can alter the levels of specific PUFA<sub>s</sub>, or derivatives thereof, found in plant parts and/or plant tissues. The elongase polypeptide coding region may be expressed either by itself or with other genes, in order to produce tissues and/or plant parts containing higher proportions of desired PUFA<sub>s</sub> or in which the PUFA composition more closely resembles that of human breast milk (Prieto et al., PCT publication WO 95/24494). The termination region may be derived from the 3' region of the gene from which the initiation region was obtained or from a different gene. A large number of termination regions are known to and have been found to be satisfactory in a variety of hosts from the same and different genera and species. The termination region usually is selected as a matter of convenience rather than because of any particular property.

As noted above, a plant (e.g., Glycine max (soybean) or Brassica napus (canola)), plant cell, plant tissue, corn, potatoe, sunflower, safflower or flax may also be utilized as a host or host cell, respectively, for expression of the elongase enzyme(s) which may, in turn, be utilized in the production of polyunsaturated fatty acids. More specifically, desired PUFA<sub>s</sub> can be expressed in seed. Methods of isolating seed oils are known in the art. Thus, in addition to providing a source for PUFA<sub>s</sub>, seed oil components may be manipulated through the expression of the elongase genes, as well as perhaps desaturase genes, in order to provide seed oils that can be added to nutritional compositions, pharmaceutical compositions, animal feeds and cosmetics. Once again, a vector which comprises a DNA sequence encoding the elongase operably linked to a promoter, will be introduced into the plant tissue or plant for a time and under conditions sufficient for expression of the elongase gene. The vector may also comprise one or more genes which encode

other enzymes, for example,  $\Delta_4$ -desaturase,  $\Delta_5$ -desaturase,  $\Delta_6$ -desaturase,  $\Delta_8$ -desaturase,  $\Delta_9$ -desaturase,  $\Delta_{10}$ -desaturase,  $\Delta_{11}$ -desaturase,  $\Delta_{13}$ -desaturase,  $\Delta_{15}$ -desaturase,  $\Delta_{17}$ -desaturase and/or  $\Delta_{19}$ -desaturase. The plant tissue or plant may produce the relevant substrate (e.g., DGLA, GLA, STA, AA, ADA, EPA, 20:4n-3, etc.) upon which the enzymes act or a vector encoding enzymes which produce such substrates may be introduced into the plant tissue, plant cell, plant, or host cell of interest. In addition, substrate may be sprayed on plant tissues expressing the appropriate enzymes. Using these various techniques, one may produce PUFA's (e.g., n-6 unsaturated fatty acids such as EPA or DHA) by use of DGLA, AA or ADA, or n-3 fatty acids such as EPA or DHA) by use of a plant cell, plant tissue, plant, or host cell of interest. It should also be noted that the invention also encompasses a transgenic plant comprising the above-described vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in, for example, the seeds of the transgenic plant.

The substrates which may be produced by the host cell either naturally or transgenically, as well as the enzymes which may be encoded by DNA sequences present in the vector, which is subsequently introduced into the host cell, are shown in Figure 1. In view of the above, the present invention also encompasses a method of producing one of the elongase enzymes described above comprising the steps of: 1) isolating the desired nucleotide sequence of the elongase cDNA; 2) constructing a vector comprising said nucleotide sequence; and 3) introducing said vector into a host cell under time and conditions sufficient for the production of the elongase enzyme.

The present invention also encompasses a method of producing polyunsaturated fatty acids comprising exposing an acid to the elongase(s) produced as above such that the elongase converts the acid to a polyunsaturated fatty acid. For example, when GLA is exposed to elongase, it is converted to DGLA. DGLA may then be exposed to  $\Delta_5$ -desaturase which converts the DGLA to AA. The AA may then be converted to EPA by use of  $\Delta_{17}$ -desaturase which may be, in turn, converted to DHA by use of elongase and a  $\Delta_4$ -desaturase. Alternatively, elongase may be utilized to convert 18:4n-3 to 20:4n-3 which may be exposed to  $\Delta_5$ -desaturase and converted to EPA. Elongase may also be used to convert 18:3n-3 to 20:3n-3, which may be, in turn, converted to 20:4n-3 by a  $\Delta_8$ -desaturase. Thus, elongase may be used in the production of polyunsaturated fatty acids which may be used, in turn, for particular beneficial purposes. (See Figure 1 for an illustration of the many critical roles the elongase enzyme plays in several biosynthetic pathways.)

Uses of the Elongase Gene and Enzyme Encoded Thereby

As noted above, the isolated elongase cDNAs and the corresponding elongase enzymes (or purified polypeptides) encoded thereby have many uses. For example, each cDNA and corresponding enzyme may be used indirectly or directly in the production of polyunsaturated fatty acids, for example, DGLA, AA, ADA, 20:4n-3 or EPA. ("Directly" is meant to encompass the situation where the enzyme directly converts the acid to another acid, the latter of which is utilized in a composition (e.g., the conversion of GLA to DGLA). "Indirectly" is meant to encompass the situation where a fatty acid is converted to another fatty acid (i.e., a pathway intermediate) by elongase

20 either naturally or transgenically, as well as the enzymes which may be encoded by DNA sequences present in the vector, which is subsequently introduced into the host cell, are shown in Figure 1. In view of the above, the present invention also encompasses a method of producing one of the elongase enzymes described above comprising the steps of: 1) isolating the desired nucleotide sequence of the elongase cDNA; 2) constructing a vector comprising said nucleotide sequence; and 3) introducing said vector into a host cell under time and conditions sufficient for the production of the elongase enzyme.

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(e.g., GLA to DGLA) and then the latter fatty acid is converted to another fatty acid by use of a non-elongase enzyme (e.g., DGLA to AA by  $\Delta 5$ -desaturase). These polyunsaturated fatty acids (i.e., those produced either directly or indirectly by activity of the elongase enzyme) may be added to, for example, nutritional compositions, pharmaceutical compositions, cosmetics, and animal feeds, all of which are encompassed by the present invention. These uses are described, in detail, below.

10 Nutritional Compositions

The present invention includes nutritional compositions. Such compositions, for purposes of the present invention, include any food or preparation for human consumption including for enteral or parenteral consumption, which when taken into the body (a) serve to nourish or build up tissues or supply energy and/or (b) maintain, restore or support adequate nutritional status or metabolic function.

The nutritional composition of the present invention comprises at least one oil or acid produced by use of at least one elongase enzyme, produced using the respective elongase gene, and may either be in a solid or liquid form. Additionally, the composition may include edible macronutrients, vitamins and minerals in amounts desired for a particular use. The amount of such ingredients will vary depending on whether the composition is intended for use with normal, healthy infants, children or adults having specialized needs such as those which accompany certain metabolic conditions (e.g., metabolic disorders).

Examples of macronutrients which may be added to the composition include but are not limited to edible fats,

carbohydrates and proteins. Examples of such edible fats include but are not limited to coconut oil, soy oil, and mono- and diglycerides. Examples of such carbohydrates include but are not limited to glucose, edible lactose and hydrolyzed starch. Additionally, examples of proteins which may be utilized in the nutritional composition of the invention include but are not limited to soy proteins, electrodialysed whey, electrodialysed skim milk, milk whey, or the hydrolysates of these proteins.

10 With respect to vitamins and minerals, the following may be added to the nutritional compositions of the present invention: calcium, phosphorus, potassium, sodium, chloride, magnesium, manganese, iron, copper, zinc, selenium, iodine, and Vitamins A, B, D, C, and the B complex. Other such vitamins and minerals may also be added.

15 The components utilized in the nutritional compositions of the present invention will be of semi-purified or purified origin. By semi-purified or purified is meant a material which has been prepared by purification of a natural material or by synthesis.

20 Examples of nutritional compositions of the present invention include but are not limited to infant formulas, dietary supplements, dietary substitutes, and rehydration compositions. Nutritional compositions of particular interest include but are not limited to those utilized for enteral and parenteral supplementation for infants, specialist infant formulae, supplements for the elderly, and supplements for those with gastrointestinal difficulties and/or malabsorption.

25 The nutritional composition of the present invention may also be added to food even when supplementation of the diet is not required. For example, the composition may be added to food

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of any type including but not limited to margarines, modified butters, cheeses, milk, yogurt, chocolate, candy, snacks, salad oils, cooking oils, cooking fats, meats, fish and beverages.

In a preferred embodiment of the present invention, the nutritional composition is an enteral nutritional product, more preferably, an adult or pediatric enteral nutritional product. This composition may be administered to adults or children experiencing stress or having specialized needs due to chronic or acute disease states. The composition may comprise, in addition to polyunsaturated fatty acids produced in accordance with the present invention, macronutrients, vitamins and minerals as described above. The macronutrients may be present in amounts equivalent to those present in human milk or on an energy basis, i.e., on a per calorie basis.

Methods for formulating liquid or solid enteral and parenteral nutritional formulas are well known in the art. (See also the Examples below.)

The enteral formula, for example, may be sterilized and subsequently utilized on a ready-to-feed (RTF) basis or stored in a concentrated liquid or powder. The powder can be prepared by spray drying the formula prepared as indicated above, and reconstituting it by rehydrating the concentrate. Adult and pediatric nutritional formulas are well known in the art and are commercially available (e.g., Similac®, Ensure®, Jevity® and Alimentum® from Ross Products Division, Abbott Laboratories, Columbus, Ohio). An oil or fatty acid produced in accordance with the present invention may be added to any of these formulas.

The energy density of the nutritional compositions of the present invention, when in liquid form, may range from about 0.6 Kcal to about 3 Kcal per ml. When in solid or powdered form,

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the nutritional supplements may contain from about 1.2 to more than 9 Kcals per gram, preferably about 3 to 7 Kcals per gm. In general, the osmolality of a liquid product should be less than 700 mOsm and, more preferably, less than 660 mOsm.

The nutritional formula may include macronutrients, vitamins, and minerals, as noted above, in addition to the PUFA's produced in accordance with the present invention. The presence of these additional components helps the individual ingest the minimum daily requirements of these elements. In addition to the provision of PUFA's, it may also be desirable to add zinc, copper, folic acid and antioxidants to the composition. It is believed that these substance boost a stressed immune system and will therefore provide further benefits to the individual receiving the composition. A pharmaceutical composition may also be supplemented with these elements.

In a more preferred embodiment, the nutritional composition comprises, in addition to antioxidants and at least one PUFA, a source of carbohydrate wherein at least 5 weight % of the carbohydrate is indigestible oligosaccharide. In a more preferred embodiment, the nutritional composition additionally comprises protein, taurine, and carnitine.

As noted above, the PUFA's produced in accordance with the present invention, or derivatives thereof, may be added to a dietary substitute or supplement, particularly an infant formula, for patients undergoing intravenous feeding or for preventing or treating malnutrition or other conditions or disease states. As background, it should be noted that human breast milk has a fatty acid profile comprising from about 0.15% to about 0.36% as DHA, from about 0.03% to about 0.13% as EPA, from about 0.30% to about 0.88% as AA, from about 0.22% to about 0.67% as DGLA, and from about 0.27% to about 1.04% as GLA. Thus,

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fatty acids such as DGLA, AA, EPA and/or docosahexaenoic acid (DHA), produced in accordance with the present invention, can be used to alter, for example, the composition of infant formulas in order to better replicate the PUFA content of human breast milk or to alter the presence of PUFAs normally found in a non-human mammal's milk. In particular, a composition for use in a pharmacologic or food supplement, particularly a breast milk substitute or supplement, will preferably comprise one or more of AA, DGLA and GLA. More preferably, the oil blend will comprise from about 0.3 to 30% AA, from about 0.2 to 30% DGLA, and/or from about 0.2 to about 30% GLA.

Parenteral nutritional compositions comprising from about 2 to about 30 weight percent fatty acids calculated as triglycerides are encompassed by the present invention. The preferred composition has about 1 to about 25 weight percent of the total PUFA composition as GLA (U.S. Patent No. 5,196,198). Other vitamins, particularly fat-soluble vitamins such as vitamin A, D, E and L-carnitine can optionally be included.

When desired, a preservative such as alpha-tocopherol may be added in an amount of about 0.1% by weight.

In addition, the ratios of AA, DGLA and GLA can be adapted for a particular given end use. When formulated as a breast milk supplement or substitute, a composition which comprises one or more of AA, DGLA and GLA will be provided in a ratio of about 1:19:30 to about 6:1:0.2, respectively. For example, the breast milk of animals can vary in ratios of AA:DGLA:GLA ranging from 1:19:30 to 6:1:0.2, which includes intermediate ratios which are preferably about 1:1:1, 1:2:1, 1:1:4. When produced together in a host cell, adjusting the rate and percent of conversion of a precursor substrate such as GLA and DGLA to AA can be used to precisely control the PUFA ratios. For example, a 5% to 10%

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conversion rate of DGLA to AA can be used to produce an AA to DGLA ratio of about 1:19, whereas a conversion rate of about 75% to 80% can be used to produce an AA to DGLA ratio of about 6:1. Therefore, whether in a cell culture system or in a host animal, regulating the timing, extent and specificity of elongase expression, as well as the expression of other desaturases, can be used to modulate PUFA levels and ratios. The PUFAs/acid(s) produced in accordance with the present invention (e.g., AA and DGLA) may then be combined with other PUFAs/acid(s) (e.g., GLA) in the desired concentrations and ratios.

Additionally, PUFA produced in accordance with the present invention or host cells containing them may also be used as animal food supplements to alter an animal's tissue or milk fatty acid composition to one more desirable for human or animal consumption.

Pharmaceutical Compositions

The present invention also encompasses a pharmaceutical composition comprising one or more of the fatty acids and/or resulting oils produced using at least one of the elongase cDNAs (i.e., MAELO, GLELO, HSELO1, CEELO, MELO4 and MELO7), in accordance with the methods described herein. More specifically, such a pharmaceutical composition may comprise one or more of the acids and/or oils as well as a standard, well-known, non-toxic pharmaceutically acceptable carrier, adjuvant or vehicle such as, for example, phosphate buffered saline, water, ethanol, polyols, vegetable oils, a wetting agent or an emulsion such as a water/oil emulsion. The composition may be in either a liquid or solid form. For example, the composition may be in the form of a tablet, capsule, ingestible liquid or

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powder, injectible, or topical ointment or cream. Proper fluidity can be maintained, for example, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. It may also be desirable to include isotonic agents, for example, sugars, sodium chloride and the like. Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening agents, flavoring agents and perfuming agents.

10 Suspensions, in addition to the active compounds, may comprise suspending agents such as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth or mixtures of these substances.

15 Solid dosage forms such as tablets and capsules can be prepared using techniques well known in the art. For example, PUFAs produced in accordance with the present invention can be tableted with conventional tablet bases such as lactose, sucrose, and cornstarch in combination with binders such as acacia, cornstarch or gelatin, disintegrating agents such as potato starch or alginic acid, and a lubricant such as stearic acid or magnesium stearate. Capsules can be prepared by incorporating these excipients into a gelatin capsule along with antioxidants and the relevant PUFA(s). The antioxidant and PUFA components should fit within the guidelines presented above.

20 For intravenous administration, the PUFAs produced in accordance with the present invention or derivatives thereof may be incorporated into commercial formulations such as Intralipids™. The typical normal adult plasma fatty acid profile comprises 6.64 to 9.46% of AA, 1.45 to 3.11% of DGLA,

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and 0.02 to 0.08% of GLA. These PUFAs or their metabolic precursors can be administered alone or in combination with other PUFAs in order to achieve a normal fatty acid profile in a patient. Where desired, the individual components of the formulations may be provided individually, in kit form, for single or multiple use. A typical dosage of a particular fatty acid is from 0.1 mg to 20 g (up to 100 g) daily and is preferably from 10 mg to 1, 2, 5 or 10 g daily.

Possible routes of administration of the pharmaceutical compositions of the present invention include, for example, enteral (e.g., oral and rectal) and parenteral. For example, a liquid preparation may be administered, for example, orally or rectally. Additionally, a homogenous mixture can be completely dispersed in water, admixed under sterile conditions with physiologically acceptable diluents, preservatives, buffers or propellants in order to form a spray or inhalant.

10 The route of administration will, of course, depend upon the desired effect. For example, if the composition is being utilized to treat rough, dry, or aging skin, to treat injured or 15 burned skin, or to treat skin or hair affected by a disease or condition, it may perhaps be applied topically.

The dosage of the composition to be administered to the patient may be determined by one of ordinary skill in the art and depends upon various factors such as weight of the patient, 20 age of the patient, immune status of the patient, etc.

With respect to form, the composition may be, for example, a solution, a dispersion, a suspension, a powder which is then reconstituted.

25 The present invention also includes the treatment of various disorders by use of the pharmaceutical and/or nutritional compositions described herein. In particular, the

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compositions of the present invention may be used to treat restenosis after angioplasty. Furthermore, symptoms of inflammation, rheumatoid arthritis, asthma and psoriasis may also be treated with the compositions of the invention.

Evidence also indicates that PUFAs may be involved in calcium metabolism; thus, the compositions of the present invention may, perhaps, be utilized in the treatment or prevention of osteoporosis and of kidney or urinary tract stones.

Additionally, the compositions of the present invention may also be used in the treatment of cancer. Malignant cells have been shown to have altered fatty acid compositions. Addition of fatty acids has been shown to slow their growth, cause cell death and increase their susceptibility to chemotherapeutic agents. Moreover, the compositions of the present invention may also be useful for treating cachexia associated with cancer. The compositions of the present invention may also be used to treat diabetes (see U.S. Patent No. 4,826,877 and Horrobin et al., Am. J. Clin. Nutr. Vol. 57 (Suppl.) 732S-737S). Altered fatty acid metabolism and composition have been demonstrated in diabetic animals.

Furthermore, the compositions of the present invention, comprising PUFAs produced either directly or indirectly through the use of the elongase enzyme(s), may also be used in the treatment of eczema, in the reduction of blood pressure, and in the improvement of mathematics examination scores.

Additionally, the compositions of the present invention may be used in inhibition of platelet aggregation, induction of vasodilation, reduction in cholesterol levels, inhibition of proliferation of vessel wall smooth muscle and fibrous tissue (Brenner et al., Adv. Exp. Med. Biol. Vol. 83, p.85-101, 1976), reduction or prevention of gastrointestinal bleeding and other

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side effects of non-steroidal anti-inflammatory drugs (see U.S. Patent No. 4,666,701), prevention or treatment of endometriosis and premenstrual syndrome (see U.S. Patent No. 4,758,592), and treatment of myalgic encephalomyelitis and chronic fatigue after viral infections (see U.S. Patent No. 5,116,871).

Further uses of the compositions of the present invention include use in the treatment of AIDS, multiple sclerosis, and inflammatory skin disorders, as well as for maintenance of general health.

Additionally, the composition of the present invention may be utilized for cosmetic purposes. It may be added to pre-existing cosmetic compositions such that a mixture is formed or may be used as a sole composition.

#### 15 Veterinary Applications

It should be noted that the above-described pharmaceutical and nutritional compositions may be utilized in connection with animals (i.e., domestic or non-domestic), as well as humans, as animals experience many of the same needs and conditions as humans. For example, the oil or acids of the present invention may be utilized in animal feed supplements, animal feed substitutes, animal vitamins or in animal topical ointments.

The present invention may be illustrated by the use of the following non-limiting examples:

#### Example I

##### Determination of Codon Usage in *Mortierella alpina*

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The 5' end of 1000 random cDNA clones were sequenced from Mortierella alpina cDNA library. The sequences were translated in six reading frames using GCG (Genetics Computer Group (Madison, Wisconsin)) with the FastA algorithm (Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85:2444-2448 (1988)) to search for similarity between a query sequence and a group of sequences of the same type (nucleic acid or protein), specifically with the Swissprot database (GeneBio, Geneva, Switzerland). Many of the clones were identified as a putative housekeeping gene based on protein sequence homology to known genes. Twenty-one M. alpina cDNA sequences which matched with known, housekeeping genes in the database were selected (see Table 1 below). M. alpina codon bias table (see Table 2) was generated based on these 21 sequences as well as the full length M. alpina  $\Delta 5$ - (see Figure 18),  $\Delta 6$ -, and  $\Delta 12$ -desaturase sequences. Since the FastA alignment between the putative protein coded by the M. alpina cDNA sequence and the known protein sequence was weak in some areas, only the codons from areas of strong homology were used.

|     |  |      |
|-----|--|------|
| 132 | 60S ribosomal protein L3-2                                   | 11   |
| 198 | Histone H3   | 9    |
| 286 | 6-phosphogluconate dehydrogenase, decarboxylating            | 11   |
| 283 | 40S ribosomal protein S22                                    | 8    |
| 127 | Elongation factor 2  | 7    |
| 197 | Actin, gamma   | 8    |
| 496 | 40S ribosomal protein S16                                    | 9    |
| 338 | Histone H4   | 7    |
| 262 | Ubiquitin  | 7    |
| 188 | Guanine nucleotide-binding protein beta subunit-like protein | 7    |
| 81  | Ubiquitin  | 7    |
|     | <b>TOTAL</b>   | 21   |
|     |  | 6252 |

Table 2

| Amino acid | Codon Bias | % used | Amino acid | Codon Bias | % used |
|------------|------------|--------|------------|------------|--------|
| Ala        | GCC        | 63%    | Lys        | AAG        | 98%    |
| Arg        | CGC        | 50%    | Met        | ATG        | 100%   |
| Asn        | AAC        | 97%    | Phs        | TTC        | 78%    |
| Asp        | GAC        | 65%    | Pro        | CCC        | 68%    |
| Cys        | TGC        | 87%    | Ser        | TCC        | 46%    |
| Gln        | CAG        | 78%    | Thr        | ACC        | 78%    |
| Glu        | GAG        | 85%    | Trp        | TGG        | 100%   |
| Gly        | GGT        | 47%    | Tyr        | TAC        | 95%    |
| His        | CAC        | 91%    | Val        | GTC        | 72%    |
| Ile        | ATC        | 72%    | Stop       | TAA        | 50%    |
|            |            |        |            | CTC        | 100%   |

Table 1

| Clone # | Match                                 | # of bp | # of |
|---------|---------------------------------------|---------|------|
| 193     | Elongation factor 1-alpha             | 426     | 14   |
| 143     | 60S ribosomal protein L17             | 417     | 15   |
| 235     | Acin1                                 | 360     | 12   |
| 299     | 40S ribosomal protein Y311            | 387     | 12   |
| 390     | Ras-related protein rab-1a            | 342     | 11   |
| 65      | 40S ribosomal protein RP10            | 366     | 12   |
| 289     | Ubiquitin-conjugating enzyme E2-16 KD | 294     | 9    |
| 151     | Ubiquinol-cytochrome C reductase      | 375     | 12   |
| 80      | Initiation factor 5A-2                | 183     | 6    |
| 33      | 60S ribosomal protein L15             | 252     | 8    |

Example II  
Cloning of a Full-length Elongase  
alpina

The  $\beta$ -ketoacyl-coenzyme A synthase (KCS) from jojoba 1  
9 and the Saccharomyces cerevisiae elongase (ELO2) were 2  
10 aligned to determine an area of amino acid homology (see 3  
11 Figure 2). The codon bias was applied to the area of 4  
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sequence corresponding to the homologous amino acids between the two elongases, and primers were designed based on this biased sequence (see Figure 3). The cDNA was excised from the M11 M. alpina cDNA library (Knutzon et al., J. Biol. Chem. 273:29350-29366 (1998)), which contains approximately  $6 \times 10^5$  clones with an average insert size of 1.1 Kb. The excised cDNA was amplified with internal primer RO339 (5' -TTG GAG AGG AGG AAG CGA CCA CCG AAG ATG ATG- 3') and a vector forward primer RO317 (5' -CAC ACA GGA AAC AGC TAT GAC CAT GAT TAC G - 3'). Polymerase Chain Reaction (PCR) was carried out in a 100  $\mu$ l volume containing: 300 ng of excised M. alpina cDNA library, 50 pmole each primer, 10  $\mu$ l of 10X buffer, 1  $\mu$ l 10 mM PCR Nucleotide Mix (Boehringer Mannheim Corp., Indianapolis, IN) and 1.0 U of Tag Polymerase. Thermocycler conditions in Perkin Elmer 9600 (Norwalk, CT) were as follows: 94°C for 2 mins., then 30 cycles of 94°C for 1 min., 58°C for 2 mins., and 72°C for 3 mins. PCR was followed by an additional extension at 72°C for 7 minutes.

The PCR amplified product was run on a gel, an amplified fragment of approximately 360 bp was gel purified, and the isolated fragment was directly sequenced using ABI 373A DNA Sequencer (Perkin Elmer, Foster City, CA). The sequence analysis package of GCG was used to compare the obtained sequence with known sequences. The sequence was translated in all six reading frames in the GCG Analysis Program using the FastA algorithm (Pearson and Lipman, supra). The Swissprot database (GeneBio, Geneva, Switzerland) of proteins was

searched. This translated cDNA fragment was identified as a part of a putative elongase based on the homology of the putative protein sequence to the S. cerevisiae ELO2 (GNS1), having 41.3% identity in 63 amino acids.

5 New primers were designed based on the putative elongase sequence and the vector, pZL1 (Life Technologies, Inc., Gaithersburg, MD) sequence used to construct M. alpina cDNA library. The M. alpina excised cDNA library was PCR amplified again using primers RO350 (5' -CAT CTC ATG GAT CCG CCA TGG CCG CAA TCT TG- 3'), 10 which has an added BamH1 restriction site (underlined), and the vector reverse primer RO352 (5' -ACG CGT ACG TAA AGC TTG- 3') to isolate the full length M. alpina elongase cDNA, using previously described conditions.

15 The termini of the approximately 1.5 Kb PCR amplified fragment was filled-in with T4 DNA polymerase (Boehringer Mannheim Corp., Indianapolis, IN) to create blunt ends and cloned into the PCR-blunt vector (Invitrogen Corp., Carlsbad, CA). This resulted in two clones, PRAE-1 and PRAE-2 (see Figure 4A). (Plasmid DNA PRAE-2 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, on August 28, 1998, under the terms of the Budapest Treaty, and was accorded deposit number ATCC 203166.) The 20 elongase cDNAs from these vectors were cut out as an EcoRI fragment and cloned into the EcoRI digested PYX242 (Novagen, Madison, WI) vector. The clones PRAE-5 and PRAE-6 (see Figure 4B) have the elongase cDNAs from PRAE-1 and PRAE-2, respectively. (Plasmid DNA PRAE-5 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-

2209, on August 28, 1990, under the terms of the Budapest Treaty, and was accorded deposit number ATCC 203167.) The sequencing of PRAE-5 and PRAE-6 revealed that 5' untranslated region of the elongase gene in PRAE-5 is 16 bp shorter than that in PRAE-6 (see Figure 5). The complete *M. alpina* elongase cDNA sequence, designated MAELO was obtained from PRAE-2 (see Figure 6). Figure 7 is the amino acid sequence obtained from the translation of MAELO. The Swissprot database (GeneBio, Geneva, Switzerland) was searched again, as previously described, with the translated MAELO: MAELO has 44.3% identity in 317 amino acids with *S. cerevisiae* GNS1 (ELO2) and 44.7% identity in 318 amino acids with *S. cerevisiae* SUR4 (ELO3). The FastA alignment among the three elongases is shown in Figure 8. At the nucleotide level (see Figure 9), MAELO has 57.4% identity in 549 bp overlap with *S. cerevisiae* GNS1 (ELO2) (GenBank Accession # S78824). However, the identity between the complete MAELO gene of 954 bp and *S. cerevisiae* GNS1 (ELO2) is 33.0%.

15 The elongase activity results from different elongases is shown in Figure 8. At the nucleotide level (see Figure 9), MAELO has 57.4% identity in 549 bp overlap with *S. cerevisiae* GNS1 (ELO2) (GenBank Accession # S78824). However, the identity between the complete MAELO gene of 954 bp and *S. cerevisiae* GNS1 (ELO2) is 33.0%.

and that in jojoba KCS was oleic acid (OA). The negative control strain was *S. cerevisiae* 334 containing PYX242 vector. The cultures were grown for 40-48 hours at 25°C, in selective media (Ausubel et al., Short Protocols in Molecular Biology, Ch. 13, p. 3-5 (1992)), in the presence of a particular substrate. The expression of the jojoba KCS gene cloned in PYES2 was under the control of GAL1 promoter, while the promoter in PYX242 is TPI, which is constitutive. Hence, the 334 (pCGN7875) and 10 334 (PYES2) cultures were induced with galactose. The GC-FAME analysis of the lipid fraction of each cell pellet was performed as previously described (Knutzon et al., supra).

15 The elongase activity results from different experiments are provided in Figure 10A and 10B. The jojoba KCS elongates long chain monounsaturated fatty acids 18:1n-9 to 20:1n-9. The amino acid homology between the *M. alpina* elongase (MAELO) and the *S. cerevisiae* ELO2 and ELO3 suggested that the proteins encoded by these genes may have similar substrate specificity. The activity of the *M. alpina* elongase, elongation (MAELO) of long chain monounsaturated and saturated fatty acids, is seen in the conversion of 18:1n-9 to 20:1n-9 and also in the synthesis of 24:0. 20 The control strain, 334 (PYX242) has very little or no detectable amount of 20:1 and 24:0 (see Figure 10A). *M. alpina* elongase (MAELO) also acts on at least one PUFA, converting 18:3n-6 (GLA) to 20:3n-6 (DGLA). The percentage of the 20:3n-6 in total lipid is higher in the strain 25 334 (PRAE-5) and 334 (PRAE-6) with the *M. alpina* elongase (MAELO) cDNA when compared to that in the control

### Example III Expression of *M. alpina* Elongase cDNA in Baker's Yeast

25 The constructs PRAE-5, and PRAE-6 were transformed into *S. cerevisiae* 334 (Hoveland et al., Gene 83:57-64 (1999)) and screened for elongase activity. The plasmid pCGN7875 (Calgene LLC, Davis, CA) containing jojoba KCS gene in PYES2 vector (Invitrogen Corp., Carlsbad, CA) was used as a positive control. The substrate used to detect 30 elongase activity in *M. alpina* elongase (MAELO) was GLA (MAELO) cDNA when compared to that in the control

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334 (pYX242). The percentages of 20:3n-6 produced were 0.092% for 334 (pYX242) vs. 0.324% for 334 (PRAE-5) and 0.269% for 334 (PRAE-6) (shown in parenthesis in Figures 10A and 10B). This difference in the fatty acid profile is also seen in the total amount of 20:3n-6 produced. Only 0.226  $\mu$ g of 20:3n-6 was produced by 334 (pYX242) while 334 (PRAE-5) and 334 (PRAE-6) produced 2.504  $\mu$ g of 20:3n-6 and 1.006  $\mu$ g of 20:3n-6, respectively. Also, when no substrate is added, the level of 20:3n-6 is not detectable.

Once 20:3n-6 is generated by the M. alpina elongase (MAELO), the  $\Delta 5$ -desaturase can convert it to AA in the desired expression system. To test this hypothesis, the constructs PRAE-5 and pGCR-4 (a  $\Delta 5$ -desaturase containing plasmid) were co-transformed into S. cerevisiae 334 and screened for AA production. The substrate used was 25  $\mu$ M GLA (18:3n-6). If the M. alpina elongase (MAELO) is active in yeast, then the substrate will be converted to DGLA (20:3n-6), which the  $\Delta 5$ -desaturase will convert to AA (20:4n-6). The results in Figure 11 confirm the production of AA and therefore, the activity of the M. alpina elongase (MAELO).

The expression of  $\Delta 5$ -,  $\Delta 6$ -, and  $\Delta 12$ -desaturases, in yeast, along with the elongase, should result in the production of AA (see Figure 1) without the need for an exogenous supply of fatty acids.

#### Example IV

A Comparison of the Expression of M. alpina Elongase cDNA MAELO and S. cerevisiae Elongase ELO2 in Baker's Yeast

The ELO2 gene encoding for the yeast elongase was cloned from an S. cerevisiae genomic library (Origens, Rockville, MD) using the primers R0514 (5' -GGC TAT CGA TCC ATG AAT TCA CTC GTC ACT CAA TAT G-3') and R0515 (5' -CCT GCC AAG CTT TTA CCT TT TCT TCT GTG TTG AG-3') incorporating the restriction sites (underlined) BamHI and HindIII (respectively). The ELO2 gene was cloned into the vector pYX242 at the BamHI and HindIII sites, designated pRELO, transformed into the S. cerevisiae host 334 (Hoveland et al., supra) and screened for PUFA elongase activity. The vector plasmid was used as a negative control and 334 (PRAE-5) was grown to compare the PUFA elongase activity. The cultures were grown as previously described with no galactose in the media and 25  $\mu$ M GLA added as a substrate. Figure 12 shows that amount of 20:3n-6 or DGLA produced (elongated from 18:3n-6 or GLA) by 334 (PRAE-5) was approximately 4 times the negative control containing the unaltered vector pYX242, while the two individual clones 334 (pRELO-1) and 334 (pRELO-2) were only twice the negative control. Additionally, when DGLA produced is expressed as a percent of the total lipids (shown in parenthesis, Figure 12), the clones 334 (pRELO-1) and 334 (pRELO-2) produced 0.153% and 0.2% DGLA respectively, while 334 (pYX242) produced 0.185% DGLA. Hence all these strains produced comparable percentages of DGLA. The strain 334 (PRAE-5), however, produced 0.279% DGLA, an increase of 50.8% over 334 (pYX242) (negative control). These data show that the S. cerevisiae elongase gene ELO2, even when overexpressed in yeast, does not elongate GLA to DGLA effectively. The M. alpina PUFA elongase activity is specific for this conversion as evidenced by the higher amount of DGLA produced compared to the control, 334 (pYX242).

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Example V  
Identification of Elongases from Other Sources Using MAELO

The TFASTA algorithm (Pearson and Lipman, *supra*) is used to search for similarity between a query peptide sequence and the database DNA sequence translated in each of the six reading frames. Translated MAELO was used as the query for a TFASTA search in GCG with the GenEMBL database (6/98) from GCG to identify other potential elongase sequences based on their amino acid similarity comparisons to translated MAELO. For example, in Figures 13 and 14, two alignments are shown between translations of two different *C. elegans* sequences from chromosome III and MAELO. *C. elegans* DNA sequence (GenBank accession # Z68749) was annotated denoting similarity with GNS1 (EL02), while the additional *C. elegans* DNA sequence (GenBank accession # U61954) was noted as similar to both GNS1 and SUR4 (EL03). These are spliced DNA fragments in which the introns have been removed from the genomic sequence, and the exons assembled and translated. The amount of amino acid identity between the putative PUFA elongases from *C. elegans* and translated MAELO are around 30%. This would point towards a common function in the fatty acid metabolism, e. g., a PUFA elongase. Figure 15 is another example of a translated *C. elegans* sequence (GenBank accession # AF003134) from chromosome III. The DNA sequence was identified that had DNA homology to the *S. cerevisiae* ELO2. Further inspection of this DNA sequence and its amino acid translation determined that there was homology to translated MAELO. *C. elegans*, therefore, may contain a PUFA elongase.

Figure 16 shows the alignments of translated DNA sequences from mouse and human, respectively, with translated MAELO. The

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mouse sequence CIG30, GenBank accession # U97107, was isolated from brown adipose tissue and reported as being "similar to yeast SUR4 protein". As shown in Figure 16, amino acids numbered 130 to 152 in the U97107 translation contain a high degree of similarity to the translated MAELO. The human sequence, GenBank accession # AC004050, from chromosome 4 was from an HTGS (High Throughput Genome Sequence). There were no annotations contained with this sequence. However, translated AC004050 had 28.7% identity in 150 amino acids with translated MAELO. This gene fragment could be a fragment of a human PUFA elongase based on its amino acid similarity to translated MAELO.

Figure 17 shows the amino acid alignment of translated MAELO and a mammalian sequence (GenBank accession # I05465, PCT# WO 88/ 01577) which claims that the protein derived from expression of this sequence is a glycosylation inhibition factor. The amino acid identities between the two proteins, signifying that there could be related function, such as PUFA elongase activity.

These examples of other translated DNA sequences and their homology to the translated MAELO illustrate that any of the above examples could potentially be a PUFA elongase. These examples are not inclusive of all the possible elongases. However, use of MAELO or its amino acid translation as a query for database searches can identify other genes which have PUFA elongase activities.

Example VI  
M. alpina cDNA Library Screening Using A Plaque Hybridization Method

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Figure 16 shows the alignments of translated DNA sequences

from mouse and human, respectively, with translated MAELO. The

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In an effort to isolate additional PUFA elongase genes from M. alpina, a conventional plaque hybridization method was used to screen an M. alpina cDNA library made in a lambda vector. The DNA probe was generated based on MAELO nucleotide sequence and was used to screen the M7+8 M. alpina cDNA library made in a λiplex vector (Knutzon et al., J. Biol. Chem. 273:29360-29366 (1998)).

To make the DNA probe for screening the library, the MAELO cDNA was digested with *Nsp*I and *Pvu*II restriction endonucleases. Three small DNA fragments, with an average size of approximately 300 bp, were produced and used as probes. The rationale for using a mixture of fragmented MAELO cDNA was based on the assumption that there might be a common region or domain in the amino acid sequence which is conserved among various PUFA

elongases present in M. alpina. Using MAELO DNA probes, the cDNA library was screened by a plaque hybridization technique according to standard protocol (Sambrook et al., Molecular Cloning, 2<sup>nd</sup> Ed., Cold Spring Harbor, 1989).

Briefly, 50,000 primary clones were plated and transferred to nylon membranes. The membranes were denatured and hybridized with alpha <sup>32</sup>P-dCTP-labelled MAELO DNA probes overnight in the hybridization buffer which contained 20 $\times$  formamide, 0.2 $\times$  PVP, BSA, Ficoll, 0.1 $\times$  SDS and 0.5 M NaCl. The filters were washed with 0.5X SSC at 37 °C and exposed to X-ray film for autoradiography. This procedure was repeated three times. Four clones (designated as F1, F2, F3, and F4) which hybridized repeatedly were picked and suspended in SM buffer (Sambrook et al., *supra*) containing 7% DMSO.

The largest open reading frame of each candidate was subcloned into yeast expression vector PYX242 (Novagen, Inc., Madison, Wisconsin). The cDNA clones F1 and F3 were subcloned

into PYX242 at the EcoRI site while F2 and F4 were subcloned at *Nco*I/*Hind*III sites. The recombinant PYX242 containing each candidate was transformed into SC334 (Noveland et al., *supra*) for expression in yeast. To determine the elongase activity, as well as substrate specificity, SC334 containing each cDNA clone was grown in minimal media lacking leucine in the presence of 25  $\mu$ M of GLA substrate as described in Example III. The fatty acid analysis was performed as described in Knutzon et al. (J. Biol. Chem. 273:29360-29366 (1998)). The results indicated that none of these four cDNA clones showed any significant activity in converting GLA to DGLA. Thus, the hybridization approach appeared to be unsuccessful in identifying additional PUFA elongases.

Example VII  
Construction of Direct cDNA Expression Library of *M. alpina* in yeast

To identify PUFA elongase genes other than MAELO, a different approach was taken to screen the M. alpina cDNA library. In particular, since Baker's yeast is incapable of producing long chain PUFAs due to the absence of respective desaturases and elongases, an attempt was made to construct an expression cDNA library of M. alpina in Saccharomyces cerevisiae. The vector PYE82 (Novagen, Inc., Madison, Wisconsin), containing the GAL4 promoter, was chosen for the expression of cDNA library in S. cerevisiae.

The conventional way by which a cDNA library is made (i.e. transformation of cDNA/vector ligated DNA mixture into host cells) is difficult in yeast because the transformation efficiency by direct electroporation of ligated DNA mix is very

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low compared to the efficiency of purified supercoiled plasmid DNA. However, the major advantage of this method is to avoid amplification of primary clones which happens when the library is made in *E. coli* as an intermediate. Due to the limitation in the number of colonies to be screened, it was decided to first optimize the efficiency of transformation in different *S. cerevisiae* strains using cDNA/vector ligated mix. The best results were obtained with a yield of  $4.5 \times 10^5$  transformants per  $\mu\text{g}$  of ligated DNA in *S. cerevisiae* strain SC334 (Noveland et al., 10 supra).

To make a direct *M. alpina* cDNA expression library in yeast total RNA was isolated from the fungus. *M. alpina* fungus (ATCC # 32221) was plated onto cornmeal agar (Difco Laboratories, Detroit, MI) and grown at room temperature for 3-4 days. Once 15 fungus growth was visible, it was inoculated into 50 ml of potato dextrose broth and shaken at room temperature very slowly to formulate spores. Once spores were visible, the 50 ml culture was inoculated into a 1 liter culture of potato dextrose, and spores were grown for 72 hours. After filtering 20 through sterile gauze, the cells were immediately frozen into liquid nitrogen for future RNA extraction. Total RNA was prepared from 36 g of cell pellet using the hot phenol/LiCl extraction method (Sambrook et al., supra). The cell pellets were homogenized in a 10 mM EDTA, 1% SDS and 200 mM sodium 25 acetate, pH 4.8 solution. Phenol and chloroform were added to the homogenates, and the aqueous layer was extracted. The aqueous layer was back extracted one more time with phenol and chloroform. Then an equal volume of 4 M lithium chloride was added. The samples were ethanol precipitated on ice for 3 hours, and pellets were obtained by centrifugation. The RNA 30 pellets were washed with 70% ethanol and resuspended in DEPC

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treated water. Total RNA was quantitated by spectrophotometry and visualized by agarose gel electrophoresis to confirm the presence of 28S and 18S ribosomal bands. Approximately, 15 mg of total RNA were obtained from 36 gram of cell pellet.

5 The library was constructed according to the standard protocol (Sambrook et. al., Molecular Cloning, 2<sup>nd</sup> Ed., Cold Spring Harbor, 1989). Messenger RNA was prepared from the total RNA using oligo dT cellulose affinity purification. Messenger RNA was reverse transcribed with oligo dT primer containing a 10 XbaI restriction site using AMV reverse transcriptase.

Following first strand cDNA synthesis, the second strand of cDNA was synthesized by adding *E. coli* DNA polymerase, *E. coli* DNA ligase and RNase H.

The EcoRI adaptor was ligated into the blunt-ended cDNA by 15 T4 DNA ligase. The cDNA sample was kinased using T4 polynucleotide kinase and digested with XbaI, diluted with column buffer and passed through a Sephacryl S-400 column. The DNA samples were eluted by high salt buffer. Samples containing DNA from 400-5,000 bps were pooled and used for ligation into a 20 pYES2 vector (Invitrogen Corp., Carlsbad, CA). The cDNA was ligated into the EcoRI/XbaI digested pYES2 vector using T4 DNA ligase. A large scale ligation reaction was carried out since a large amount of the ligated DNA (2-3  $\mu\text{g}$ ) is required in direct transformation of yeast.

25 To transform yeast cells directly with the cDNA/pYES2 ligated mixture, competent SC334 cells were prepared using the LiAC TRAFO method (Gietz et. al., Mol. Cell. Biol., 5: 255-269, 1995). Briefly, fresh culture of SC334 from the plate was inoculated into 50 ml YPD medium. The culture was grown at 30 °C with shaking until the OD at 600 had reached 1.0. Thirty ml of this starter was inoculated into 300 ml of YPD liquid medium and

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incubated with shaking until the cell number of the culture reached ~  $3.5 \times 10^6$  cell/ml (approximately 3-4 h). The cells were harvested and washed with sterile water. The entire cell pellet was resuspended in 1.5 ml of freshly prepared 1X TE/LiAC (0.1M LiAc). These cells were used immediately for the transformations.

Seven hundred and fifty microliters of competent SC334 cells were aliquoted into 15 ml falcon tubes. Approximately 2 ug of cDNA/pYES2 ligated DNA were added to the cells along with 10 carrier DNA and mixed gently. Three milliliters of sterile 40% PEG/LiAC was added to the cells and mixed gently but thoroughly. The cells were incubated at 30 °C for 30 min with shaking and subsequently given heat shock at 42 °C for 15 min. The cells were cooled, pelleted, and resuspended in 5 ml of 1X TE. A 100  $\mu$ l aliquot of the above cells was plated onto fifty 150 mm selective agar plates lacking uracil (Ausubel et al., supra) and incubated at 30 °C for 3 days. A total of  $8 \times 10^5$  primary clones were obtained. Five colonies were pooled in 1 ml minimal media lacking uracil (Ausubel et al., supra) and glycerol added to 15  $\mu$ l aliquot of the above cells. A total of 5,000 pools were made for screening.

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were plated onto selective agar plates, as described in Example VII, to obtain individual colonies. Forty different yeast colonies were randomly picked, and each colony was inoculated into 5 ml of selective liquid medium lacking uracil (as described in Example VII) and grown, while shaking, for 24 hours at 30 °C. Plasmid DNA was extracted from these colonies by the bead beating method (Hoffman et al., Gene 57:267 (1987) adapted as follows:

Pellets from 5 ml of culture were lysed in 0.5 ml of a 100 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA and 0.1% SDS solution. Sterile 0.5 mm glass beads of equal volume were added and manually vortexed for 3 minutes. Two hundred microliters of the same buffer were added, and the mixture was vortexed for an additional minute. The samples were centrifuged on high for 2 minutes, and cytoplasmic extract was then transferred to a fresh tube. An equal volume of phenol/CHCl<sub>3</sub> was added to the sample, vortexed and centrifuged again for 2 minutes. The aqueous layer was re-extracted twice and precipitated with 0.3 M sodium acetate and approximately 2.5 volumes of ethanol for 30 minutes at -20 °C. The precipitates were washed with 70% ethanol and resuspended in water. To eliminate RNA and any protein contamination, the plasmid DNAs isolated from 40 different samples were further purified using the QIAprep Spin Miniprep Kit according to the manufacturer's protocol (Qiagen Inc.,

25 Valencia, CA). The plasmid DNA samples were then restricted with EcoRI and XbaI restriction endonucleases to release the cDNA fragment, and the digest was analyzed on 1% agarose gel.

The results indicated that the majority of the cDNAs of the direct library varied in length from 0.8 Kb to 1.5 Kb.

30 To screen the library, the glycerol stocks were thawed and approximately 0.5 ml was added to 5 ml of liquid selective media

#### Example VIII MAD (M. alpina Direct) Screening in Yeast

25 The quality of the library was analyzed by determining the average size of the cDNAs in the library. Since the screening of the library was based on the expression of the cDNA, it was important to determine the average size of the cDNA present in the library. The expression library containing the longest cDNAs would be the best appropriate choice to isolate full-length cDNAs of interest. To this end, randomly selected pools

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lacking uracil (Ausubel et al., *supra*) and grown at 30°C for 24 hours. The culture was then transferred into 50 ml of liquid selective medium lacking uracil with 2% galactose and 25  $\mu$ M GLA (substrate for the elongase enzyme) for 24 hours at 25 °C with shaking. The GC-FAME analysis of the lipid content in the cell pellet of each induced culture was performed as previously described (Knutzon et al., *supra*). The MAELO (PRAE-5 in PYX242 grown in selective media lacking leucine) was used as a positive control in each batch run. MAELO had consistently been able to convert 1.5% of GLA to DGLA (see Example III).

#### Example IX

##### Identification of a cDNA Encoding a Potential PUFA Elongase

After screening and analyzing approximately 750 individual pools by GC-FAME analysis, as described in Example VIII, one pool of five colonies (i.e., MAD708) appeared to have significant enzymatic activity in converting GLA to DGLA. This activity was found to be approximately 5 fold higher than the *M. alpina* elongase activity (MAELO) in terms of DGLA/GLA ratio (Figure 19). This pool was tested again under identical assay conditions to confirm the initial findings. The repeat experiment showed 9.5% conversion of GLA to DGLA and was again around 5 fold higher than *M. alpina* elongase activity (MAELO). These results strongly indicated that the MAD708 pool contained an elongase candidate which was specific for GLA as substrate. Since MAD708 was a pool of five different clones, it was necessary to isolate the individual cDNA clone which encoded for elongase activity from this pool. To do this, the original MAD708 glycerol stock was plated onto a selective media agar

plate lacking uracil (Ausubel et al., *supra*). Thirty individual colonies were picked and grown in liquid selective medium, lacking uracil with 2% galactose, as previously described in Example VIII, in the presence of GLA. The cell pellet obtained from each culture was then subjected to fatty GC-FAME analysis (Knutzon et al., *supra*) along with a positive control of 334 (PRAE-5) (MAELO in PYX242). The fatty acid analysis from the 30 individual clones from the MAD708 expression pool in yeast revealed that 5 of the 30 clones showed elongase activity in converting GLA to DGLA. The fatty acid profiles of the active clones MAD708-2, MAD708-10, MAD708-18, MAD708-19 and MAD708-30 are shown in Figure 20. As shown in this Figure, MAD708-2, 10, and 30 produced the most DGLA, approximately 25 fold more than MAELO (PRAE-5). These 3 converted in the range of 41% to 49% of GLA to DGLA. Other clones, MAD708-18 and MAD708-19, converted 8% and 21% of GLA to DGLA, respectively. All MAD708 clones converted a higher percentage of GLA to DGLA with respect to MAELO encoded elongase (3.4%).

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#### Characterization of cDNAs Encoding Elongase

Plasmid DNA was extracted from SC334 yeast clones (MAD708 pool) that showed significant GLA specific elongase activity by the bead beating method, as described in Example VIII. To determine the size of the cDNA insert, PCR was performed using each plasmid DNA obtained from positive elongase clones as a template. The forward primer R0541 (5'- GAC TAC TAG CAG CTG TAA TAC -3') and the reverse primer R0540 (5'- GTG ATT GTA AGC GTG ACA TAA -3') are in the multicloning site of the pYES2 vector

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plate lacking uracil (Ausubel et al., *supra*). Thirty individual colonies were picked and grown in liquid selective medium, lacking uracil with 2% galactose, as previously described in Example VIII, in the presence of GLA. The cell pellet obtained from each culture was then subjected to fatty GC-FAME analysis (Knutzon et al., *supra*) along with a positive control of 334 (PRAE-5) (MAELO in PYX242). The fatty acid analysis from the 30 individual clones from the MAD708 expression pool in yeast revealed that 5 of the 30 clones showed elongase activity in converting GLA to DGLA. The fatty acid profiles of the active clones MAD708-2, MAD708-10, MAD708-18, MAD708-19 and MAD708-30 are shown in Figure 20. As shown in this Figure, MAD708-2, 10, and 30 produced the most DGLA, approximately 25 fold more than MAELO (PRAE-5). These 3 converted in the range of 41% to 49% of GLA to DGLA. Other clones, MAD708-18 and MAD708-19, converted 8% and 21% of GLA to DGLA, respectively. All MAD708 clones converted a higher percentage of GLA to DGLA with respect to MAELO encoded elongase (3.4%).

#### Example X

##### Characterization of cDNAs Encoding Elongase

Plasmid DNA was extracted from SC334 yeast clones (MAD708 pool) that showed significant GLA specific elongase activity by the bead beating method, as described in Example VIII. To determine the size of the cDNA insert, PCR was performed using each plasmid DNA obtained from positive elongase clones as a template. The forward primer R0541 (5'- GAC TAC TAG CAG CTG TAA TAC -3') and the reverse primer R0540 (5'- GTG ATT GTA AGC GTG ACA TAA -3') are in the multicloning site of the pYES2 vector

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and were used to amplify the cDNA insert within the EcoRI and XbaI sites. PCR reaction was performed in a 50  $\mu$ l volume containing 4  $\mu$ l of plasmid DNA, 50 pmole of each primer, 5  $\mu$ l of 10X buffer, 1  $\mu$ l 10  $\mu$ M PCR Nucleotide Mix (Boehringer Mannheim Corp., Indianapolis, IN) and 0.5  $\mu$ l of High Five Tag polymerase (Boehringer Mannheim, Indianapolis, IN). The amplification was carried out as follows: 2 mins. denaturation at 94 °C, then 94 °C for 1 min., 55 °C for 2 mins., and 72 °C for 3 mins. for 30 cycles, and 7 mins. extension at 72 °C at the end of the amplification. Analysis of PCR amplified products on a 1% agarose gel showed the sizes of the elongase cDNAs to be around 1.0 - 1.2 Kb. The plasmid DNAs, containing the potential elongase cDNAs, were designated as pRPB2, pRPB10, pRPB18, pRPB19, and pRPB30. Since the cDNA library was made in the pYES2 vector at the EcoRI and XbaI sites, the size of the cDNA present in each plasmid was further confirmed by digesting the above plasmids with EcoRI and XbaI.

The plasmid DNAs isolated from yeast were re-amplified in E. coli for long-term storage of the cDNA clones as well as for DNA sequencing. E. coli TOP10 (Invitrogen Corp., Carlsbad, CA) cells were transformed with the pRPB recombinant plasmids according to the manufacturer's protocol. The transformants obtained from each plasmid DNA were inoculated into LB containing ampicillin (50  $\mu$ g/ml) and grown overnight at 37 °C with shaking. Plasmid DNAs were isolated from these cultures by using QIAprep Spin Miniprep (Qiagen Inc., Valencia, CA) according to the manufacturer's protocol. The purified plasmid DNAs were then used for sequencing from both 5' and 3' ends. The DNA sequencing was performed by using a 373A Stretch ABI automated DNA sequencer (Perkin Elmer, Foster City, CA) according to the manufacturer's protocol. Primers used for

sequencing were the forward primer R0541 (5' - GAT TAC TAG CAG CTG TAA TAC -3') and the reverse primer R0540 (5' - GTG AAT GTA AGC GTG ACA TAA -3') contained in the multicloning sites of the pYES2 vector. The obtained nucleotide sequences were transferred to Sequencher software program (Gene Codes Corporation, Ann Arbor, MI) for analysis. The DNA sequence analysis revealed that all five elongase cDNAs contained the identical nucleotide sequence with a common overlap of 301 nucleotides. Each DNA sequence contains a putative start site 10 at the beginning of the 5' end and a stop codon with poly A tail at the end of the 3' site. To further confirm the DNA sequence, internal forward primers R0728 (5' - GAG ACT TTG AGC GGT TCG -3') and R0730 (5' - TCT CTG CTG CGT TGA ACT CG -3'), along with reverse primers R0729 (5' - AAA GCT CTT GAC CTC GAA C 15 -3') and R0731 (5' - AAC TTG ATG AAC GAC ACG TG -3') were designed within the cDNA, and used for sequencing of pRPB2, since this candidate possessed the highest elongase activity. The entire nucleotide sequence was analyzed by the Sequencher program (Figure 21), and the longest open reading frame deduced 20 from the entire cDNA sequence in pRPB2 appeared to be 957 bp in length (Figure 22). The deduced open reading frame was then translated into the corresponding amino acid sequence, and the predicted sequence is shown in Figure 23. The elongase encoded by the cDNA (pRPB2) identified from M. alpina appears to be a 25 318 amino acid long protein which is nearly identical in size with translated MABLO. This new elongase cDNA was designated as "GLELO" and its encoded protein has been named "GLE elongase".

Plasmid DNA pRPB2 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209 on July 22, 1999 under the terms of the Budapest Treaty. It was accorded ATCC Deposit # PTA-402.

Example XI  
Biochemical Characterization of GLA Elongase (GLELO)

A. Confirmation of GLA Elongase Activity

To further confirm the activity of the GLA elongase encoded by the pRB2 recombinant plasmid, elongase activity screening was repeated on the yeast clone SC334 containing pRB2 plasmid. This experiment was also conducted to assure consistent lipid extraction and to detect the activity of GLA elongase by averaging four independent experiments. The S. cerevisiae 334 glycerol stock containing pRB2 was plated onto minimal media agar plates lacking uracil. Individual colonies were randomly picked and grown in minimal medium lacking uracil, as described in Example VIII. The four independent cultures were combined, and a 5 ml aliquot was used as an inoculum for four separate 50 ml cultures. The cultures were then grown in the presence of GLA and were subjected to fatty acid analysis along with a negative control of S. cerevisiae 334 containing pYES2, as described in Example VIII. The average elongase activity from four independent cultures of 334 (pRB2) with 25  $\mu$ M GLA is shown in Figure 24. The GLA elongase activity of each of the four independent samples of 334 (pRB2) appeared to be consistent with an average conversion of 62% GLA to DGLA.

B. Determination of GLELO Substrate Specificity for GLA Elongase

To analyze the substrate specificity of the GLA elongase, the culture of 334 (pRB2) was tested with different fatty acid substrates besides GLA (e.g., SA(18:0), OA(18:1), LA(18:2n-6), AA(20:4n-6), ADA(22:4n-6), ALA(18:3n-3), STA(18:4n-3), and EPA(20:5n-3)). Under identical assay conditions, the only other substrate utilized by the elongase enzyme was STA, a fatty acid

from the n-3 pathway. GLA elongase was able to convert 73% of STA to 20:4n-3 (Figure 25). From these experiments, it can be concluded that the GLA elongase has substrate specificity for both GLA and STA, indicating that it possesses elongase activity along both the n-6 and n-3 pathways.

C. Co-expression of Fungal GLELO and  $\Delta 5$ -Desaturase Gene in Yeast

Once DGLA (20:3n-6) is produced by the GLA elongase, the  $\Delta 5$ -desaturase can convert it to AA (20:4n-6) in a desired co-expression system. This scheme, as depicted in Figure 1, can be tested by co-transforming S. cerevisiae 334 with plasmids pRB2 and pRPE31 (the recombinant plasmid pYX242 containing a  $\Delta 5$ -desaturase cDNA (Figure 18) cloned at the EcoRI site. The co-transformed yeast cultures were supplemented with 25  $\mu$ M GLA and analyzed for AA synthesis. If both elongase and  $\Delta 5$ -desaturase enzymes are expressed, the GLA substrate will be converted to DGLA, which will then be converted to AA. The results in Figure 26A indicate that the sequential action of GLA elongase and  $\Delta 5$ -desaturase on GLA substrate resulted in an average conversion of 20.27% GLA to AA. Therefore, the GLA elongase has the ability to work with other enzymes in the n-6 PUFA synthetic pathway to produce desirable fatty acids.

To determine whether the above conversion is also true in n-3 pathways, the similar co-expression experiments were carried out in the presence of 25  $\mu$ M STA. Again, if both enzymes are expressed, the STA substrate will be converted to 20:4n-3 which will then be converted to EPA (20:5n-3) by the  $\Delta 5$ -desaturase. Figure 26B shows the results in which the production of EPA (approx. 40%) is observed. Once again, the GLA elongase

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demonstrates its ability to work with Δ5-desaturase in the n-3 pathway to produce desirable fatty acids.

Example XII  
5 Sequence Comparison Between GLELO and Other Fungal Elongases

The sequence analysis package of GCG (see Example I) was used to compare the GLELO sequence with known protein sequences. The nucleotide sequence of GLELO open reading frame was first 10 translated into amino acid sequence that was used as a query sequence to search Swissprot database (see Example I) using the FastA algorithm (see Example I). Based on amino acid sequence similarity, the best matches were found with S. cerevisiae YJR6 (an EST with unknown annotation) with 33.9% identity in 189 (an EST with unknown annotation) with 33.9% identity in 189 15 amino acid overlap, S. cerevisiae ELO2 (GNS1) with 25.8% identity in 295 amino acid overlap, and S. cerevisiae ELO (S0RA) with 25.2% identity in 313 amino acid overlap. The FastA alignment of GLELO with MAELO showed 30.9% identity in 275 amino acids (Figure 27). GCG Pileup program creates a multiple 20 sequence alignment from a group of related sequences using progressive, pairwise alignments (see Example I), and was used with the elongases described above. The Pileup results indicate that there are many conserved regions among the elongases including a putative histidine box, which is underlined (Knutzon et al., J. Biol. Chem. 273: 29360-29366, 1998) (Figure 28). 25 Thus, although GLELO has similarity with MAELO, the difference in their encoded elongases may presumably be due to their substrate preference. GLA elongase can convert a higher percentage of GLA to DGLA than M. alpina elongase. In addition, MAELO expression in S. cerevisiae showed elongation of saturated 30 elongases in S. cerevisiae showed elongation of saturated

and monounsaturated fatty acids in addition to GLA elongation to DGLA (see Example III).

Example XIII  
5 Identification of *M. alpina* MAELO Homologues in Mammals

The MAELO translated sequence was used to search the Unified Human Transcript Database of Abbott Laboratories, 100 10 Abbott Park Rd., Abbott Park, Illinois 60064. This database was searched using Basic Local Alignment Search Tool (BLAST) (Altschul et al., Nuc. Acids Res. 25:3389-3402 (1997)) which is a set of similarity search programs designed to explore all of the available sequence databases regardless of whether the query 15 is a protein or DNA." Specifically, the blastn algorithm was used (i.e., a protein query search to a nucleotide database translated in six reading frames). The contig (CC) sequences in the Unified Human Transcript Database are consensus sequences representing groups of expressed sequence tags (EST) cDNAs 20 derived from the public domain and from the Incyte LIFESEQ™ database of ESTs (Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, CA 94304) that are clustered together on the basis of defined sequence homology, and assembled on the basis of sequence overlap. Two sequences from this database, 25 CC067284R1 and CC148548T1 had 28% identity in 242 amino acid overlap and 28.6% identity in 266 amino acid overlap, respectively, with the translated MAELO sequence. The two derived and edited sequences were designated as hs1 and hs2, respectively, and copied into the sequence analysis software package of GCG (see Example I). The translated MAELO sequence 30 was aligned with translated HS1 (28.5% identity in 242 amino

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acid(s) and HS2 (28.2% identity in 266 amino acids) cDNA sequences using the FastA algorithm, as shown in Figures 29 and 30, respectively. HS1 cDNA nucleotide sequence also had 86.9% identity in 844 bp with the 105465 nucleotide sequence (see Example V). The translated HS2 cDNA sequence had 100% identity with the amino acid sequence from GenBank with accession number W74824 (see published PCR application W09839448).

The National Center for Biotechnology Information (NCBI) at <http://www.ncbi.nlm.nih.gov/> was used to conduct database searches using tblastn with the 28 amino acid sequence (DTIIFILRKQKLIFQWYHHTVLLYSW) translated from AC04050 (a human sequence identified in a TFASTA search, see Example V). This amino acid sequence contains a histidine box (underlined), which has a noted motif of desaturases (Knutzon et al., supra), and both PUFA elongases, MAELO and GLELO (see Figure 2B). A translated mouse sequence shown previously in Example V (GenBank Accession #U97107) and a translated *C. elegans* sequence (GenBank Accession # U41011) had the highest matches with this 28 amino acid query. The NCBI mouse EST database was searched again with tblastn, using translated U41011 as a query. An additional mouse sequence was identified (GenBank Accession #AF014033.1), annotated as "putative involvement in fatty acid elongation." Three longer sequences (GenBank Accession #'s AA591034, AA89549, and AA839346) were identified through a tblastn search of the mouse EST database with translated AF014033.1 and combined into one sequence designated as mm2. The FastA alignment (see Example I) of translated mm2 and MAELO is shown in Figure 31. Another related, but not identical mouse sequence (GenBank Accession #AF225632), was also identified in a tblastn search of the mouse EST database with AF014033.1. The FastA alignment with translated AF225632 to MAELO is shown in Figure 30

32. The percent identity for both translated MM2 and AI 225632 with translated MAELO is 30.4% in 191 and 115 amino acid overlap, respectively. The level of amino acid identity with translated MAELO with these two translated mouse sequences identifies them as putative homologues of PUFA elongases.

10 The TFASTA algorithm, which compares a protein sequence to the database DNA sequence translated in each of the six reading frames, was used with translated GLELO as the query. The GenEMBL database from GCG was used to identify other potential 15 elongase sequences based on their amino acid similarity to translated GLELO. Three human sequences were found to have matches with the GLELO amino acid sequence. These sequences have GenBank accession numbers 1) AI15960, 2) AI034374, and 3) AC004030. AI15960, a  *Homo sapien* EST sequence, has 40.3% 20 identity in 144 amino acid overlap with translated GLELO (see Figure 33). A translated region of the human genomic sequence AI034374, derived from chromosome VI has 46.7% identity in a 60 amino acid overlap with translated GLELO. This homologous region in AI034374 appeared to be a part of the HS1 amino acid 25 sequence which was shown to have homology with translated MAELO (see Example XIII). Therefore, HS1 sequence has similarity with both MAELO (see Figure 29) as well as GLELO (see Figure 34). A translated region of a human genomic sequence AC004050 from chromosome IV has 34.8% identity in 89 amino acid overlap with 30 translated GLELO (see Figure 35). The amino acid identities between GLELO and these human sequences indicate that the

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Example XIV  
Identification of *M. alpina* GLELO Homologues in Mammals

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proteins derived from these human sequences could have related function, such as PUFAs elongase activity.

To identify a mouse cDNA similar to GLELO, TFASTA searches were performed with the GenEMBL database using translated GLELO as a query. From the TFASTA searches, the three mouse sequences with the highest matches to translated GLELO were identified: (GenBank accession numbers 1) AF104033, 2) AI595258, and 3) U97107. AF104033 is annotated as "MUEL protein having putative fatty acid elongase with homology to yeast ELO3 (SUR4)" and is a part of the sequence of MM2. The MM2 sequence was initially derived from AF104033 mouse sequence, but the entire MM2 sequence was finally obtained through further mouse EST database searches and also shown to have homology with translated MAELO (see Example XIII and Figure 31). When this MM2 amino acid sequence was aligned with translated GLELO sequence using FastA, a 34.6% identity in 211 amino acid overlap was found (see Figure 36) indicating that MM2 also has homology with GLELO. AI595258 is a mouse cDNA clone having 5' similarity with yeast ELO3 elongase and is part of mouse EST cDNA AI225632. The AI225632 mouse sequence, which is a longer sequence than AI595258, was shown to have similarity with translated MAELO (see Figure 32). The AI225632 was also aligned with the translated GLELO, and the FastA alignment is shown in Figure 37. A 35.3% identity in 199 amino acid overlap has been found. The third sequence, U97107, a mouse sequence, was annotated as "similar to yeast ELO3 (SUR4) gene." The FastA alignment of translated GLELO with U97107 is shown in Figure 38 where a 23.7% identity in 279 amino acid overlap was found. Previously, a region of U97107 was also found to have a high degree of homology with MAELO based on a FastA alignment (see Example V and Figure 16).

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The above searches clearly indicate that the same human and mouse sequences were obtained by using either MAELO or GLELO as a query.

Example XV

5 Identification of *M. alpina* GLELO and MAELO Homologues in Other PUFAs Producing Organisms

A) *Caenorhabditis elegans*:

10 A putative amino acid sequence deduced from a chromosomal sequence of *C. elegans* (GenBank Accession # U41011) was able to identify a partial sequence contained in the mouse MM2 putative PUFAs elongase which has amino acid similarity with both GLELO elongase (GLELO) and *M. alpina* elongase (MAELO). It was therefore conceivable that *C. elegans* homologues of GLELO or MAELO might be present in the nematode database. The putative amino acid sequences derived from GLELO and MAELO sequences were used as queries independently to search the nematode databases. A BLAST search (see Example XIII) was performed on wormpep16

15 (blastp compares an amino acid query sequence against a nucleotide sequence database) and wormpep 16cDNAs (tblastn) databases which are predicted proteins and cDNAs obtained from the *C. elegans* genome sequencing project or EST's and their corresponding cDNA sequences, respectively. These sequence data were produced by the *C. elegans* Sequencing group, carried out jointly by the Sanger Centre and Genome Sequencing Center, and can be obtained from <ftp://ftp.sanger.ac.uk/pub/databases/wormpep/>. At least seven putative *C. elegans* translated sequences were identified by their amino acid sequence homology to the translated amino acid sequence of both GLELO and MAELO. The GenBank Accession #'s of those genomic

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Sequences containing the deduced amino acids were identified as Z19154, U68749 (2 deduced proteins (F56H11.4 and F56H11.3 (wormpep Accession #'s)), U41011, U61954 (2 deduced proteins (F41H10.7 and F41H10.8 (wormpep Accession #'s)), and Z81058. Those underlined were identified in a previous search using translated MAELO as query (see Example V). As an example, the FASTA amino acid alignments of translated U68749 (F56H11.4) with translated GLELO and MAELO are shown in Figures 39 and 40. Translated U68749 (F56H11.4) has 25-30% identity with both M. alpina elongase and GLA elongase in approximately a 200 amino acid overlap (see Figures 39 and 40). For all seven translated putative C. elegans cDNAs, the FastA alignments to translated GLELO was between 25-30% identity in a 200 amino acid overlap while the identity was 26-34% in at least a 188 amino acid overlap for translated MAELO. The alignment similarities indicate that either translated GLELO or MAELO can be used to identify potential genes from C. elegans with elongase activity.

B) Drosophila melanogaster:

The translated deduced cDNA from the genomic sequence U41011 (C. elegans) had its highest match with a Drosophila melanogaster EST, accession number AI134173 in a blastn search (compares a nucleotide query sequence against a nucleotide database) of the "other ESTs" database through NCBI (see Example XIII) and was assembled with an overlapping DNA EST fragment, accession number AI517255. The translated DNA fragment DM1, derived from the two overlapping sequences was aligned with translated GLELO as well as MAELO (see Figures 41 and 42) using FastA in GCG (see Example I). The alignments showed 27.2% identity with GLA elongase in a 206 amino acid overlap and 30% identity with M. alpina elongase in a 237 amino acid overlap.

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Thus, based on amino acid similarity, the DM1 could be a potential homologue to GLELO or MAELO having PUFA elongase-like activity. Moreover, using DNA sequences of GLELO and MAELO as queries for database searches, homologues with PUFA elongase activity from Drosophila can be identified.

Example XVI

Cloning and Expression of A Human PUFA Elongase Homologue

Many potential PUFA elongase sequences were identified based on their amino acid similarities to translated GLELO and/or MAELO. To determine the potential elongase activities of these sequences, the cDNA encoding the full-length protein is then identified, cloned, and expressed, as demonstrated in the present example.

Primers R0719 (5' -GGT TCT CCC GAT TTT GAT GCA TC-3') and R0720 (5' -GGT TTC AAA GCT TTG ACT TCA ATC CTT CCG-3') were designed based on the putative HSL sequence, and used to amplify the human liver Marathon-Ready cDNA (Clontech Laboratories, Inc., Palo Alto, California). The polymerase Chain Reaction (PCR) was carried out in a 50  $\mu$ l volume containing: 5  $\mu$ l of human liver Marathon-Ready cDNA, 50 pmole each primer, 1  $\mu$ l 10 mM PCR Nucleotide Mix (Boehringer Mannheim Corp., Indianapolis, IN), 5  $\mu$ l 10 X buffer and 1.0 U of Advantage 25 KlenTaq Polymerase Mix (Clontech Laboratories, Inc., Palo Alto, CA). Thermocycler conditions in Perkin Elmer 9600 (Norwalk, CT) were as follows: 94 °C for 2 mins, then 30 cycles of 94 °C for 1 min., 58 °C for 2 mins, and 72 °C for 3 mins. PCR was followed by an additional extension cycle at 72 °C for 7 minutes.

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The PCR amplified product was run on a gel, an amplified fragment of approximately 960 bp was gel purified, the termini of the fragment filled-in with T4 DNA polymerase (Boehringer Mannheim, Corp., Indianapolis, IN), and cloned into PCR-Blunt Vector (Invitrogen Corp., Carlsbad, CA) following manufacturer's protocol. The new plasmid was designated as PRAE-52, and the putative PUFA elongase cDNA in this clone was sequenced using ABI 373A Stretch DNA Sequencer (Perkin Elmer, Foster City, CA). The putative PUFA elongase cDNA sequence in plasmid PRAE-52 is shown in Figure 43, and the translated sequence is shown in Figure 44.

The putative PUFA elongase cDNA from plasmid PRAE-52 was then digested with NcoI/HindIII, gel purified, and ligated into PYX242 (NcoI/HindIII). The new plasmid was designated as PRAE-58-A1. (Plasmid 58-A1 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 on August 19, 1999, under the terms of the Budapest Treaty and was accorded deposit number PTA-566.) The construct PRAE-58-A1 was transformed into *S. cerevisiae* 334 (Hoveland et al., *supra*) and screened for elongase activity. The negative control strain was *S. cerevisiae* 334 containing PYX242 vector. The cultures were grown for 24 hours at 30°C, in selective media (Ausubel et al., *supra*), in the presence of 25  $\mu$ M of GLA or AA. In this study, DGLA or arachidic acid (ADA, 22:4n-6), respectively, was the predicted product of human elongase activity. When GLA was used as a substrate, the yeast cells containing the human elongase cDNA contained elevated levels of DGLA compared to control cells, 2.75% vs. 0.09% of total fatty acids, respectively (see Figure 45). When AA was used as a substrate, the yeast cells containing the human elongase cDNA contained elevated levels of ADA compared to control cells, none

detected vs. 1.21% of total fatty acids, respectively. Thus, the human elongase converts both 18 and 20 carbon chain long PUFAs to their respective elongated fatty acids.

The yeast cells containing the human elongase cDNA also had elevated levels of monounsaturated fatty acids including 18:1n-7, 20:1n-7, 20:1n-9, and 18:1n-5, compared to the control strain. Therefore, these results indicate that the identified human elongase is capable of utilizing PUFAs as well as monounsaturated fatty acids as substrates. Thus, this human sequence HSEL01, and its encoded protein (HSEL01p), possess elongase activity independent of substrate specificity.

To further confirm the substrate specificity of the human elongation enzyme, described above and referred to herein as HSEL01, the recombinant yeast strain 334 (PRAE-58-A1) was grown in minimal media containing n-6 fatty acids GLA, AA, or n-3 fatty acids ALA, STA, or EPA. The lipid profiles of these yeast cultures, when examined by GC and GC-MS, indicated that there were accumulations of DGLA, ADA,  $\omega$ 3-eicosatrienoic acid (ETRA, C20:3n-3), ETA, and DPA, respectively (Figure 51). The levels of these fatty acids were 7.29% (DGLA), 6.26% (ADA), 6.15% (ETRA), 10.06% (ETA), and 6.66% (DPA), respectively, of the total fatty acids in the strain containing the PRAE-58-A1 sequence. These represented 78.3%, 42.7%, 30.4%, 79.2%, and 71.7% conversions of the substrate fatty acids, respectively, to the products elongated by two carbon atoms.

The yeast cells expressing the recombinant HSEL01 sequence, compared to the control cells, also contained significantly elevated levels of C18:1n-7 and C20:1n-7, and to a lesser extent, eicosenoic acid (EA, C20:1n-9) (Figure 45). This finding suggested that the recombinant HSEL01 protein (HSEL01p) might also be involved in the elongation of monounsaturated

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To further confirm the substrate specificity of the human elongation enzyme, described above and referred to herein as HSEL01, the recombinant yeast strain 334 (PRAE-58-A1) was grown in minimal media containing n-6 fatty acids GLA, AA, or n-3 fatty acids ALA, STA, or EPA. The lipid profiles of these yeast cultures, when examined by GC and GC-MS, indicated that there were accumulations of DGLA, ADA,  $\omega$ 3-eicosatrienoic acid (ETRA, C20:3n-3), ETA, and DPA, respectively (Figure 51). The levels of these fatty acids were 7.29% (DGLA), 6.26% (ADA), 6.15% (ETRA), 10.06% (ETA), and 6.66% (DPA), respectively, of the total fatty acids in the strain containing the PRAE-58-A1 sequence. These represented 78.3%, 42.7%, 30.4%, 79.2%, and 71.7% conversions of the substrate fatty acids, respectively, to the products elongated by two carbon atoms.

The yeast cells expressing the recombinant HSEL01 sequence, compared to the control cells, also contained significantly elevated levels of C18:1n-7 and C20:1n-7, and to a lesser extent, eicosenoic acid (EA, C20:1n-9) (Figure 45). This finding suggested that the recombinant HSEL01 protein (HSEL01p) might also be involved in the elongation of monounsaturated

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fatty acids of 16 or 18 carbon lengths. To confirm this hypothesis, 25  $\mu$ M of exogenous OA was added as a substrate to the recombinant yeast strain 334 (PRAE-58-A1). After incubation, the accumulation of EA at 2.25% of the total fatty acids demonstrated that the expressed HSEL01 enzyme could elongate monounsaturated fatty acids (Figure 51). However, the conversion of OA to EA by recombinant HSEL01 was only 8.9%; this conversion was significantly lower than the endogenous conversion of C16:1n-7 (to C18:1n-7) or C18:1n-7 (to C20:1n-7), which was 20.4% and 58.1%, respectively.

To determine whether the substrate concentration affects the conversion of 18 and 20 carbon fatty acids to the respective elongated products, two different concentrations of GLA, AA, and EPA were examined (Figure 52). When 25  $\mu$ M of the substrates GLA, AA, and EPA were added exogenously, the levels of the fatty acids produced by two carbon elongation were 3.95% (DGLA), 2.91% (ADA), and 4.82% (DPA), respectively, of the total fatty acids in the lysates of 334 (PRAE-58-A1). These represented 62.4%, 27.5%, and 70.3% conversions of the substrate fatty acids, respectively, to the products elongated by two carbon atoms. When 100  $\mu$ M of the substrates GLA, AA, and EPA were added, the levels of the fatty acids produced by two carbon elongation were 9.56% (DGLA), 3.90% (ADA), and 11.50% (DPA), respectively, of the total fatty acids in the lysates of 334 (PRAE-58-A1). These represented 39.8%, 15.7%, and 45.7% conversions of the substrate fatty acids, respectively, to the products elongated by two carbon atoms. Although the addition of more substrates led to higher percentages of the two carbon elongated products, the overall conversion rate decreased by at least 35%.

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To further confirm the substrate specificity of HSEL01p, the recombinant yeast strain 334 (PRAE-58-A1) was grown in minimal media containing 25  $\mu$ M of saturated, monounsaturated, or PUFAs. The lipid profiles of these various substrates revealed that HSEL01p is not involved in the elongation of saturated fatty acids such as palmitic acid (PA, C16:0), stearic acid (SA, C18:0), arachidic acid (ARA, C20:0), behenic acid (BA, C22:0) (Figure 53A). HSEL01p is also not involved in the elongation of monounsaturated fatty acids OA and EA. When PTA was added as a substrate, 12.76% of the total fatty acids was OA. However, this is not an increase in the level of OA compared to the samples where PTA was not added, as OA was 25-31% of the total fatty acids in all samples. HSEL01p is involved in the elongation of n-6 PUFAs LA, GLA, and AA, but not DGLA or ADA (Figure 53B). The lipid profiles of these yeast cultures indicated that there were accumulations of C20:2n-6, DGLA, and ADA, respectively, but not C22:3n-6 or C24:4n-6. The levels of these fatty acids were 0.74% (C20:2n-6), 2.46% (DGLA), and 2.14% (ADA), respectively, of the total fatty acids in the lysates of 334 (PRAE-58-A1). These represented 13.2%, 51.4%, and 27.1% conversions of the substrate fatty acids, respectively, to the products elongated by two carbon atoms. HSEL01p is also involved in the elongation of n-3 PUFAs ALA, STA, and EPA, but not DPA (Figure 53C). The lipid profiles of these yeast cultures indicated that there were accumulations of ETrA, ETrA, and DPA, respectively, but not C24:5n-3. The levels of these fatty acids were 1.03% ETrA, 2.24% (ETrA), and 3.19% (DPA), respectively, of the total fatty acids in the strain containing the PRAE-58-A1 sequence. These represented 22.2%, 61.9%, and 39.5% conversions of the substrate fatty acids, respectively, to the products elongated by two carbon atoms. All results

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confirmed that the expression of HSEL01 from human liver in yeast resulted in the elongation of various long-chain PUFAs in n-6 and n-3 fatty acid pathways.

**5 Cloning, Expression and Characterization of a *C. elegans* PUFA Elongase**

Several putative *C. elegans* elongases were identified with 10 amino acid homology to both translated GIELO and MABLO. As with the human cDNA sequence, cloning of a cDNA and expression in yeast was used to determine if indeed it was a PUFA elongase. Primers R0738 (5' -AAT CAG GAA TTC ATG GCT CAG CAT CCG CTC GTC CAA C -3') and R0739 (5' -CCG CTT GTC GAC TTA GTT GRT CTT CTC 15 CTT TGG CAC -3') with restriction sites EcoRI and SalI (underlined), respectively, were based on the putative cDNA sequence contained in the genomic sequence U66749 (wormpep cDNA accession #F56H11.4.) A PCR amplification was performed in a 100  $\mu$ l volume containing: 250 ng excised C. elegans library cDNA primer, 10  $\mu$ l 10X reaction buffer (Boehringer Mannheim Corp., Indianapolis, IN), 1  $\mu$ l 10 mM PCR Nucleotide mix (Boehringer Mannheim Corp., Indianapolis, IN), and 2.5 U Taq polymerase (Boehringer Mannheim Corp., Indianapolis, IN). Thermocycler conditions in a Perkin Elmer 9600 (Norwalk, CT) were as follows: 20 95 °C for 5 mins, then 25 cycles of 94 °C for 30 secs, 55 °C for 2 mins, and 72 °C for 2 mins. PCR was followed by an additional cycle of 72 °C for 7 minutes.

The PCR amplified product was purified from an agarose gel, 25 cut with EcoRI and SalI, ligated to PYX242 (Invitrogen Corp., Carlsbad, CA) (linearized with EcoRI and SalI) using the Rapid

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Ligation kit (Boehringer Mannheim Corp., Indianapolis, IN), according to the manufacturer's protocol and transformed into E. coli Top10 cells (Invitrogen Corp., Carlsbad, CA). The new plasmids, designated PRET-21 and PRET-22 (two individual clones from the ligation), were sequenced with the 373A Stretch DNA sequencer ABI (Perkin Elmer, Foster City, CA), and the cDNA sequences were identical. The 867 base cDNA nucleotide sequence of the plasmid PRET-22 containing the putative elongase is shown in Figure 46 and the translated sequence of 288 amino acids is shown in Figure 47. (Plasmid PRET-22 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 on August 19, 1999, under the terms of the Budapest Treaty and was accorded deposit number PTA-565.) The plasmids PRET-21 and -22 were transformed into S. cerevisiae 334 as previously described (see Example III) and the resulting yeast cultures (334 (PRET-21) and 334 (PRET-22)) grown 15 in 100 ml of selective media without leucine (Ausubel et al., supra) for 48 hours at 20 °C in the presence of 50  $\mu$ M GLA and AA. The cell pellets were collected and subjected to fatty acid analysis and the results shown in Figure 48. DGLA, the predicted product from GLA elongation, was found to be an average of 1.79% of the total lipid in the two samples, versus 0.13% for the negative control (334 containing plasmid PYX242) indicating that the enzyme encoded by both PRET-21 and PRET-22 20 possessed GLA elongase activity. The percent conversion of GLA to DGLA by 334 (PRET-21) and 334 (PRET-22) was 11.1% and 19.4% respectively with an average of 15.25%. Interestingly, almost no elongation of AA or any endogenous fatty acid was observed 25 (Fig. 48). These results indicate that the elongase encoded by this newly identified C. elegans cDNA, CEEL01, is able to

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specifically elongate GLA to DGLA, suggesting that it may be a *C. elegans* homologue of GLA elongase.

To further confirm the GLA elongation activity of CEELO1, the experiment described in the paragraphs above was repeated with the exception that GLA and AA were added to cultures of 334 (pRET-22) separately. Again, GLA was elongated to DGLA with a 38.2% conversion rate. No elongation activity of AA was detected as shown in Figure 61. In this case, the percent conversion appears to be double that described in previous results (see Figure 48), and may either be due to the absence of the additional substrate (AA) or the subculturing of the yeast. CEELO1 has the additional activity of elongating endogenous 16:1:n-7 to 18:1:n-7 with a 9.12% conversion rate compared to 3.9% control culture 334 (pRX242) under identical conditions. Thus, the *C. elegans* elongation enzyme possesses a major elongation activity for a C18 polyunsaturated fatty acid and a minor activity for a C16 monounsaturated fatty acid.

Additionally, to further determine the substrate specificity of CEELO1, 50  $\mu$ M of each substrate besides GLA (e.g., SA (18:0), OA (18:1), LA (18:2:n-6), DGLA (20:3:n-g), AA (20:2n-6), ADA (22:4n-6), ALA (18:3n-3), PA (18:0), EPA (20:5n-3) and STA (18:4n-2)) was added individually to cultures of 334 (pRET-22) and grown for 48 hours at 20 °C, as described in Example XVII. STA was the only exogenously added substrate that was elongated. The CEELO1 elongated 13% of STA incorporated to ETA (20:4n-3) (see Figure 62).

Parallel to Examples III and XI, the *C. elegans* CEELO1 gene in the plasmid pRET22 and *M. alpina*  $\Delta 5$  desaturase (pCGR-4; see Example III) were co-expressed in yeast to determine if AA or EPA could be produced from exogenously added GLA or STA, respectively. When a yeast culture containing both pRET22 and

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pCGR4 plasmids was grown in the presence of 50  $\mu$ M GLA or STA in media lacking leucine and uracil, the percent conversion to the final products of AA and EPA, respectively, appeared identical (27% conversion) (see Figure 63). Thus, simultaneous heterologous expression of CEELO1 and a  $\Delta 5$  desaturase results in the biosynthesis of AA and EPA from GLA and STA, respectively in yeast.

Example XVIII  
Isolation of a Putative Human Elongase cDNA Based on AC004050 Sequence

To isolate the full length putative elongase cDNA based on the AC004050 sequence, primers RP735 (5' -CCT CCT GAA TTC CAA CAC TAT TCA GCT TTC -3') and R073 (5' -TAA TAC GAC TCA CTA TAG GG -3') were used to PCR amplify the human liver Marathon-Ready cDNA (Clontech Laboratories, Inc., Palo Alto, CA). The PCR was carried out using the Advantage™ cDNA PCR Kit (Clontech Laboratories, Inc., Palo Alto, CA) with 5  $\mu$ l of human liver Marathon-Ready cDNA and 50 pmole each primer following manufacturer's instructions. Thermocycler conditions in Perkin Elmer 9600 (Norwalk, CT) were as follows: 94 °C for 2 mins, then 30 cycles of 94 °C for 1 min., 58 °C for 2 mins., and 72 °C for 3 mins. PCR was followed by an additional extension at 72 °C for 7 mins.

The PCR amplified product was run on a gel, an amplified fragment of approximately 1 Kb was gel purified, the termini of the fragment were filled in with T4DNA polymerase (Boehringer Mannheim, Corp., Carlsbad, CA) following manufacturer's instructions. The new plasmid was designated as pRAE-59, and

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30 Mannheim, Corp., Carlsbad, CA) following manufacturer's instructions. The new plasmid was designated as pRAE-59, and

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the putative PUFA elongase cDNA in this plasmid, designated as HS3, was sequenced using the ABI 373A Stretch Sequencer (Perkin Elmer, Foster City, CA). The putative PUFA elongase cDNA sequence HS3 is shown in Figure 49, and the translated sequence is shown in Figure 50.

Example XIX  
Cloning and Expression of a Mouse PUFA Elongation Enzyme

The National Center for Biotechnology Information (NCBI at <http://www.ncbi.nlm.nih.gov>) was used to conduct database searches using blastn with the mouse EST sequence AI225632 (see Example XII). Three mouse EST sequences were identified (GenBank Accession #'s AI428130, AI595258, and AA061089), and assembled to generate a putative full-length elongation enzyme sequence, designated as MELO4. Primers R0819 (5' -ATG ATG CCA TGT AGC AGC TGA AGC CCT TTG- 3') and R0820 (5' -CAG TCT CTG CTT TAA AAC AAG CTC GTC- 3') were designed based on the putative full length mouse elongation enzyme sequence, and used to amplify the mouse brain Marathon-Ready cDNA (Clontech Laboratories, Inc., Palo Alto, California). The Polymerase Chain Reaction (PCR) was carried out as previously described (Example XVI). The PCR amplified product was run on a gel, an amplified fragment of approximately 1,000 bp was gel purified, the termini of the fragment were digested with NcoI and DraI (Boehringer Mannheim, Corp., Indianapolis, IN), and the fragment was cloned into PYX242 (NcoI/HindIII). The new plasmid was designated as pRAE-84, and the putative PUFA elongation enzyme cDNA in this clone was sequenced using ABI 373A Stretch DNA Sequencer (Perkin Elmer, Foster City, CA). The putative PUFA

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elongation enzyme cDNA sequence in plasmid pRAE-84 is shown in Figure 54, and the translated sequence is shown in Figure 55.

The construct pRAE-84 was transformed into *S. cerevisiae* 334 (Hoveland et al., supra) and screened for elongase activity.

The negative control strain was *S. cerevisiae* 334 containing PYX242 vector. The cultures were grown for 42-48 hours at 30°C, in selective media (Ausubel et al., supra), in the presence of 25  $\mu$ M of GLA, AA, ADA, STA, EPA, or DPA. The lipid profiles of these yeast cultures indicated that GLA was not elongated to the expected product of DGIA. However, there were accumulations of ADA,  $\omega$ 6-tetracosatetraenoic acid (TTA, C24:4n-6), ETA, DPA, and  $\omega$ 3-tetracosapentaenoic acid (TPA, C24:5n-3), respectively (Figure 56). The n-6 fatty acid substrate AA was converted to ADA, which was subsequently converted to TTA, and the n-3 fatty acid EPA was converted to DPA, which was subsequently converted to TPA. The levels of these fatty acids were 0.64% (ADA), 1.07% (TTA), 1.47% (DPA), and 7.06% (TPA), respectively, of the total fatty acids in the strain containing the pRAE-84 sequence. These represented 10.4%, 62.6%, 32.7%, and 82.8% conversions of the substrate fatty acids, respectively, to the products elongated by two carbon atoms. The C22 substrates ADA and EPA were elongated to 2.4% (TTA) and 3.82% (TPA) of the total fatty acids. These represented 9.2% and 43.9% conversions of the substrate fatty acids, respectively. The expression of MELO4 in yeast results in the conversion of C20 and C22 fatty acids to the respective elongated products. The conversion rate of C22 to C24 fatty acids is much greater when the exogenously added substrate is C20 fatty acid.

To further confirm the substrate specificity of MELO4 protein (MELO4), the recombinant yeast strain 334 (pRAE-84) was

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grown in minimal media containing 25  $\mu$ M of saturated, monounsaturated, or polyunsaturated fatty acids. The lipid profiles of these various substrates revealed that MELO4p is not involved in the elongation of saturated fatty acids such as PA, SA, ARA, or BA (Figure 57A). MELO4p is also not involved in the elongation of monounsaturated fatty acids PTA, OA, or EA. MELO4p is involved in the elongation of n-6 PUFAs AA and ADA, but not LA or DGLA (Figure 57B). The lipid profiles of these yeast cultures indicated that there were accumulations of ADA and TTA, but not C20:2n-6 or C22:3n-6. When AA was added exogenously, the levels of product fatty acids were 0.5% (ADA) and 0.39% (TTA), and when ADA was added exogenously, the level of product fatty acid was 1.3% (TTA) of the total fatty acids in the strain containing the PRAE-84 sequence. These represented 8.7%, 43.8%, and 7.3% conversions of the substrate fatty acids, respectively, to the products elongated by two carbon atoms. MELO4p is also involved in the elongation of GLA to DGLA. The lipid profile of the strain containing the PRAE-84 sequence, in the presence of GLA, had 0.43% of DGLA, which represented 14.7% conversion of GLA to DGLA. MELO4p is also involved in the elongation of n-3 PUFAs EPA and DPA (Figure 53C). The lipid profiles of these yeast cultures indicated that there were accumulations of DPA and TPA. When EPA was added, the levels of these fatty acids were 1.21% (DPA) and 3.38% (TPA), and when DPA was added, the level of the product fatty acid was 3.09% (TPA) of the total fatty acids in the strain containing the PRAE-84 sequence. These represented 24.0%, 73.6%, and 46.4% conversions of the substrate fatty acids, respectively, to the products elongated by two carbon atoms. MELO4p is also involved in the elongation of STA to C22:4n-3. When STA was added, the levels of fatty acids produced by two-carbon elongation were 0.3% TPA

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and 0.23% C22:4n-3. These represented 11.1% and 43.4% conversions of substrate fatty acids to the products elongated by two carbon atoms. MELO4p also appeared to be involved in the elongation of ALA; however, the small amount of the fatty acid produced by two-carbon elongation (0.16% of EPA) may not be significant. All results confirmed that the expression of MELO4p from mouse brain in yeast resulted in the elongation of C20 and C22 long-chain PUFAs in n-6 and n-3 fatty acid pathways.

10 Example XX  
Identification, Cloning, and Expression of HSEL01 Homologue from  
Mouse

The National Center for Biotechnology Information (NCBI at <http://www.ncbi.nlm.nih.gov>) was used to conduct database searches using blastn with the HSEL01 sequence. Two human EST sequences were identified (GenBank Accession #'s AI787925 and AI746838) and the respective cDNA clones (I.M.A.G.E. Consortium Clone ID's 2076831 and 205182) were purchased through Research Genetics (Huntsville, AL). Primers RO833 (5' -GGT TTT ACC ATG GAA CAT TTC GAT GCG TCA C- 3') and RO832 (5' -CGA CCT GCA GCT CCA GCA CA- 3') were designed based on 5' sequence of the putative mouse elongation enzyme, and the cDNA clone vector, respectively. Primers RO833 and RO832 were used to amplify the 20 mouse cDNA clone 2076182. The Polymerase Chain Reaction (PCR) was carried out as previously described (Example XVI). The 25 termini of the PCR amplified product were filled-in with T4 DNA polymerase (Boehringer Mannheim, Corp., Indianapolis, IN) and the 5' region was digested with NcoI. The modified fragment was 30 run on a gel, an amplified fragment of approximately 2.4 Kp was gel purified, and the fragment was cloned into PYX242

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(NcoI/EcoRV). The new plasmid was designated as pRAE-87, and the putative PUFA elongation enzyme cDNA in this clone, MELO7, was sequenced using ABI 373A stretch DNA Sequencer (Perkin Elmer, Foster City, CA). The putative PUFA elongation enzyme cDNA sequence in plasmid pRAE-87 (MELO7) is shown in Figure 58, and the translated sequence is shown in Figure 59.

The construct pRAE-87 was transformed into S. cerevisiae 334 (Hoveland et al., supra) and screened for elongase activity. The negative control strain was S. cerevisiae 334 containing pYR242 vector. The cultures were grown for 42-48 hours at 30°C, in selective media (Ansuel et al., supra), in the presence of 25 μM of GLA, AA, STA, EPA, DPA, or ADA. The lipid profiles of the yeast cultures expressing MELO7 indicated that there were accumulations of DGLA, ADA, and ETA, respectively (Figure 60). The levels of these fatty acids were 4.1% (DGLA), 6.33% (ADA), 3.4% (ETA), and 6.18% (DPA), respectively, of the total fatty acids in the strain containing the pRAE-87 sequence. These represented 78.7%, 36.0%, 81.0%, and 57.4% conversions of the substrate fatty acids, respectively, to the products elongated by two carbon atoms. MELO7 protein (MELO7) was not involved in the elongation of ADA. MELO7p also appeared to be involved in further elongation of the fatty acid DPA produced by two-carbon elongation to TPA when EPA was the added substrate, and when DPA was added. However, the small amounts of the product fatty acids (0.27% and 0.25% of TPA) may not be significant.

The yeast cells expressing the recombinant MELO7 sequence, compared to the control cells, also contained significantly elevated levels of C18:1n-7 and C20:1n-7. All results confirmed that the expression of MELO7 from mouse embryo in yeast resulted in the elongation of various long-chain PUFAs in n-6 and n-3 fatty acid pathways, and that MELO7 was a homologue of HSELO1.

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Example XXI  
Cloning and Expression of a Fungal PUFA Elongation Enzyme

The translated amino acid sequences of the four elongases, HSELO1, MELO4, GLELO, and CEBLO, were aligned to identify areas of homology (Figure 64). A primer was designed based on a block of sequences that showed similarities (underlined). Primer RC0895 (5' -GTA GTC WGA GTC CAT GAT WAC GTC GAT RAA WGA GTT WAG-3') and vector primer ROB98 (5' -CCC AGT CAC GAC GTT GTC AAA CGA CCG CCA G- 3') were used to amplify the cDNA from Thraustochytrium aureum 7091 (T7091). PCR was carried out in a 50 μl volume containing: 1 μl of T7091 cDNA, 0.2 μM dNTP mix, 50 pM each primer, 5 μl of 10 X buffer, 1.5 μl of 50 mM MgSO<sub>4</sub>, and 0.5 U of Tag DNA Polymerase. Thermocycler conditions in Perkin Elmer 9600 were as follows: 94°C for 3 min, then 30 cycles of 95°C for 45 sec., 55 °C for 30 sec., and 68°C for 2 min. The PCR amplified mixture was run on a gel, an amplified fragment of approximately 750 bp was gel purified, and the isolated fragment was cloned into the PCR-blunt vector (Invitrogen, Co., Carlsbad, CA).

Twelve clones were prepared and sequenced. All twelve clones had the same sequence, and the consensus sequence was designated as cld6 (Figure 65). The translated amino acid sequence of cld6 (Figure 66) had 34.7% identity in 190 amino acids with HSELO1, 35.8% identity in 187 amino acids with MELO4, 45.6% identity in 160 amino acids with GLELO, and 32.9% identity in 155 amino acids with CEBLO. A new primer was designed based on the 5' sequence of cld6, at the first Met. RO1160 (5' -AGG GAA CCA TGG CAA ACA GCA GCG TGT GGG ATG- 3'), which has an added

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NcoI site (underlined), and vector primer RO899 (5' -AGC GGA TAA CAA TTT CAC ACA GGA AAC AGC- 3') were used to amplify the T7091 cDNA. The Polymerase Chain Reaction (PCR) was carried out as described above. The PCR amplified product was run on a gel, an amplified fragment of approximately 1.2 Kb was gel purified, the termini of the fragment were digested with NcoI and HindIII (Boehringer Mannheim, Corp., Indianapolis, IN), and the fragment was cloned into PYX242 (NcoI/HindIII). The new plasmids were designated as pRAT-4-A1, pRAT-4-A2, pRAT-4-A3, pRAT-4-A4, pRAT-4-A6, pRAT-4-A7, and pRAT-4-D1. (Plasmid DNA pRAT-4-A7 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, on June 29, 2001, under the terms of the Budapest Treaty, and was accorded deposit number ATCC PTA-3490.) The putative PUFA elongation enzyme cDNAs in these clones were sequenced using ABI 373A Stretch DNA Sequencer (Perkin Elmer, Foster City, CA). The putative PUFA elongation enzyme cDNA sequences in plasmids pRAT-4-A1 through pRAT-4-D1 are shown in Figures 67-73, and the translated sequences are shown in Figures 74-80, respectively.

The constructs pRAT-4-A1 through pRAT-4-D1 were transformed into S. cerevisiae 334 (Hoveland et al., *supra*) and screened for elongase activity. S. cerevisiae 334 containing the unaltered pYX242 vector was used as a negative control. The cultures were grown for 48 hours at 24°C, in selective media (Ausubel et al., *supra*), in the presence of 25  $\mu$ M of GLA or EPA. In this study, DGLA or  $\omega$ 3-docosapentaenoic acid (DPA, 22:5n-3), respectively, was the predicted product of the elongase activity. The lipid profiles of these yeast cultures indicated that GLA was elongated to DGLA in all the samples, except for 334 (pRAT-4-A3) (Figure 81). This was expected in that the cDNA sequence of

pRAT-4-A3 had several stops at the 5' end of the sequence, which would result in a truncated version of the translated enzyme (Figure 76). Although the DGLA level was very low for 334 (pRAT-4-A2), the GLA level was the lowest in this sample; therefore, the conversion level of the fatty acid to its elongated form was significant at 28.4%. What was not expected was that there was definite substrate specificity among the expressed enzymes. The cultures of 334 (pRAT-4-A1) and 334 (pRAT-4-A2) had very low levels of DPA when EPA was added as a substrate, indicating that the expressed enzymes in these cultures preferred the elongation of the 18-carbon chain long PUFA, and not the 20-chain long PUFA, EPA. The cultures of 334 (pRAT-4-A4), 334 (pRAT-4-A6), 334 (pRAT-4-A7), and 334 (pRAT-D1) all had significant levels of DPA present, indicating that the expressed enzymes in these cultures were involved in the elongation of both 18- and 20-carbon chain long PUFA's to their respective elongated fatty acids.

The amino acid sequences of the 6 active clones were compared to determine if the substrate preferences were dictated by the translated sequences (Figure 82). The cDNA sequences of pRAT-4-A1 and pRAT-4-A2 differed from other sequences in that they had a mutation at Y377C and V371A, respectively. This must be a critical region of the enzyme for 20-carbon chain elongation. The other sequences also had mutations, pRAT-4-A4 (I475V), pRAT-4-A6 (D26G and V458A), and pRAT-4-D1 (K182R and E269V); however, these mutations do not appear to interfere with 20-carbon chain elongation. The pRAT-4-A7 cDNA had a single, silent mutation at base 726 in comparison to the consensus sequence.

The translated amino acid sequence of the consensus TEL01 sequence had 34.0% identity in 265 amino acids with HSE101

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(Figure 83), 34.1% identity in 267 amino acids with MELO4 (Figure 84), 43.4% identity in 244 amino acids with GbELO (Figure 85), and 34.3% identity in 239 amino acids with CEELO (Figure 86).

The yeast cells containing the fungal elongase cDNAs also had elevated levels of the monounsaturated fatty acid 18:1n-7, compared to the control strain (Figure 81). Therefore, these results indicated that the identified fungal elongases are capable of utilizing PUFAs as well as monounsaturated fatty acids as substrates. Thus, these fungal sequences TEL01, and their encoded proteins (TELO1p), possess elongase activity independent of substrate specificity.

To further confirm the substrate specificity of the fungal elongation enzyme, described above and referred to herein as TEL01, the recombinant yeast strain 334 (PRAT-4-A4), 334 (PRAT-4-A6), 334 (PRAT-4-A7), and 334 (PRAT-4-D1) were grown in minimal media containing n-6 fatty acids GLA, AA, or n-3 fatty acids STA, or EPA, or no substrate. The lipid profiles of these yeast cultures, when examined by GC and GC-MS, indicated that there were accumulations of DGLA, ADA, ETA,  $\omega$ 3-docosatetraenoic acid (DTA, C22:4n-3), and DPA, respectively (Figure 87). The levels of these fatty acids were 3.88 - 5.42% (DGLA), 0.18 - 0.75% (ADA), 2.27 - 4.01% (ETA), 0.16 - 1.10% (DTA), and 0.54 - 1.74% (DPA), respectively, of the total fatty acids in the strains containing the TEL01 sequence. These represented 64.0 - 77.2%, 1.0 - 5.0%, 79.3 - 89.6%, 3.8 - 32.6%, and 3.4 - 13.3% conversions of the substrate fatty acids, respectively, to the products elongated by two carbon atoms.

The yeast cells expressing the recombinant TEL01 sequence, compared to the control cells, also contained significantly

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elevated levels of C18:1n-7 and decreased levels of C16:1n-7 (Figure 87). This finding suggested that the recombinant TEL01 protein (TELO1p) might also be involved in the elongation of monounsaturated fatty acids of 16-carbon length.

To further confirm the substrate specificity of TEL01p, the recombinant yeast strain 334 (PRAT-4-A7) was grown in minimal media containing 25  $\mu$ M of various PUFAs. The lipid profiles of these various substrates revealed that TEL01p is involved in the elongation of n-6 PUFAs LA, GLA, and AA, but not DGLA (Figure 88). The lipid profiles of these yeast cultures indicated that there were accumulations of C20:2n-6, DGLA, and ADA, respectively, but not C22:3n-6. The levels of these fatty acids were 1.07% (C20:2n-6), 5.84% (DGLA), and 0.76% (ADA), respectively, of the total fatty acids in the lysates of 15 334 (PRAT-4-A7). These represented 23.2%, 78.6%, and 3.5% conversions of the substrate fatty acids, respectively, to the products elongated by two-carbon atoms. TEL01p is also involved in the elongation of n-3 PUFAs ALA, STA, and EPA (Figure 88). The lipid profiles of these yeast cultures indicated that there were accumulations of ETA, EPA, and DPA, respectively. The levels of these fatty acids were 2.71% ETA, 4.19% (ETA), and 1.99% (DPA), respectively, of the total fatty acids in the 20 strain containing the TEL01 sequence. These represented 43.3%, 85.3%, and 11.5% conversions of the substrate fatty acids, 25 respectively, to the products elongated by two-carbon atoms. When STA was added as a substrate, there was also an accumulation of DTA in the culture. The level of this fatty acid was 0.74%, which represented 15.0% conversion of STA, the two-carbon chain elongated product from substrate STA. All 30 results confirmed that the expression of TEL01 from T7091 in

yeast resulted in the elongation of various long-chain PUFAs in n-6 and n-3 fatty acid pathways.

Nutritional Compositions

The PUFAs described in the Detailed Description may be utilized in various nutritional supplements, infant formulations, nutritional substitutes and other nutritional solutions.

10 I. INFANT FORMULATIONS

A. Isomil® Soy Formula with Iron:

Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cows milk. A feeding for patients with disorders for which lactose should be avoided: lactase deficiency, lactose intolerance and galactosemia.

Features:

-Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity.

20 -Lactose-free formulation to avoid lactose-associated diarrhea. -Low osmolarity (240 mOsm/kg water) to reduce risk of osmotic diarrhea.

25 -Dual carbohydrates (corn syrup and sucrose) designed to enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut. -1.8 mg of Iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency. -Recommended levels of vitamins and minerals.

30 -Vegetable oils to provide recommended levels of essential fatty acids. -Milk-white color, milk-like consistency and pleasant aroma.

Ingredients: (Pareve) 85% water, 4.9% corn syrup, 2.6% sugar (sucrose), 2.1% soy oil, 1.9% soy protein isolate, 1.4% coconut oil, 0.15% calcium citrate, 0.11% calcium phosphate tribasic, 5 potassium citrate, potassium phosphate monobasic, potassium chloride, mono- and disglycerides, soy lecithin, carrageenan, L-methionine, magnesium chloride, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, D-inositol, alpha-tocopherol acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, 10 biotin, sodium selenite, vitamin D3 and cyanocobalamin.

B. Isomil® DF Soy Formula For Diarrhea:

Usage: As a short-term feeding for the dietary management of diarrhea in infants and toddlers.

20 Features:

-First infant formula to contain added dietary fiber from soy fiber specifically for diarrhea management.

25 -Clinically shown to reduce the duration of loose, watery stools during mild to severe diarrhea in infants. -Nutritionally complete to meet the nutritional needs of the infant.

-Soy protein isolate with added L-methionine meets or exceeds an infant's requirement for all essential amino acids.

30 -Lactose-free formulation to avoid lactose-associated diarrhea.

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- Low osmolarity (240 mOsm/kg water) to reduce the risk of osmotic diarrhea.
- Dual carbohydrates (corn syrup and sucrose) designed to enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut.
- Meets or exceeds the vitamin and mineral levels recommended by the Committee on Nutrition of the American Academy of Pediatrics and required by the Infant Formula Act.
- 1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
- Vegetable oils to provide recommended levels of essential fatty acids.

Ingredients: (Pareve) 86% water, 4.8% corn syrup, 2.5% sugar (sucrose), 2.1% soy oil, 2.0% soy protein isolate, 1.4% coconut oil, 0.77% soy fiber, 0.12% calcium citrate, 0.11% calcium phosphate tribasic, 0.10% potassium citrate, potassium chloride, potassium phosphate monobasic, mono and diglycerides, soy lecithin, carrageenan, magnesium chloride, ascorbic acid, L-methionine, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

- Low osmolarity (240 mOsm/kg water) to reduce the risk of an allergy or sensitivity to cow's-milk protein or an intolerance to sucrose. A feeding for patients with disorders for which lactose and sucrose should be avoided.
- 5 Features:
  - Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity.
  - Lactose-free formulation to avoid lactose-associated diarrhea (carbohydrate source is Polycose® Glucose Polymers).
  - Sucrose free for the patient who cannot tolerate sucrose.
  - Low osmolarity (180 mOsm/kg water) to reduce risk of osmotic diarrhea.
  - 1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
  - 15 -Recommended levels of vitamins and minerals.
    - Vegetable oils to provide recommended levels of essential fatty acids.
    - Milk-white color, milk-like consistency and pleasant aroma.
- 20 Ingredients: (Pareve) 75% water, 11.8% hydrolyzed cornstarch, 4.1% soy oil, 4.1% soy protein isolate, 2.8% coconut oil, 1.0% modified cornstarch, 0.38% calcium phosphate tribasic, 0.17% potassium citrate, 0.13% potassium chloride, mono- and 25 diglycerides, soy lecithin, magnesium chloride, ascorbic acid, L-methionine, calcium carbonate, sodium chloride, choline chloride, carrageenan, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.
- 30 C. Isomil® SF Sucrose-Free Soy Formula With Iron:

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hydrochloride, folic acid, manganese sulfate, potassium iodide, phyllloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

**S. D. Isomil® 20 Soy Formula With Iron Ready To Feed,**

20 Cal/Fl oz.:

Usage: When a soy feeding is desired.

Ingredients: (Pareve) 85% water, 4.9% corn syrup, 2.6%

10 sugar (sucrose), 2.1% soy oil, 1.9% soy protein isolate, 1.4% coconut oil, 0.15% calcium citrate, 0.11% calcium phosphate tribasic, potassium citrate, potassium phosphate monobasic, potassium chloride, mono- and diglycerides, soy lecithin, carrageenan, L-methionine, magnesium chloride, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, niacinamide, ferrous sulfate, 15 calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, 20 vitamin D3 and cyanocobalamin.

20 folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

**E. Similac® Infant Formula:**

25

Usage: When an infant formula is needed: if the decision is made to discontinue breastfeeding before age 1 year, if a supplement to breastfeeding is needed or as a routine feeding if breastfeeding is not adopted.

30 Features:  
Features:

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-Protein of appropriate quality and quantity for good growth, heat-denatured, which reduces the risk of milk-associated enteric blood loss.

-Fat from a blend of vegetable oils (doubly homogenized),

5 providing essential linoleic acid that is easily absorbed.

-Carbohydrate as lactose in proportion similar to that of human milk.

-Low renal solute load to minimize stress on developing organs.

-Powder, Concentrated Liquid and Ready To Feed forms.

10 Ingredients: (-D) Water, nonfat milk, lactose, soy oil, coconut oil, mono- and diglycerides, soy lecithin, ascorbic acid, carrageenan, choline chloride, taurine, m-inositol, alpha-tocopheryl acetate, zinc sulfate, niacinamide, ferrous sulfate, 15 calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, 20 vitamin D3 and cyanocobalamin.

**F. Similac® NeoCare Premature Infant Formula With Iron:**

Usage: For premature infants' special nutritional needs after hospital discharge. Similac NeoCare is a nutritionally complete formula developed to

25 provide premature infants with extra calories, protein, vitamins and minerals needed to promote catch-up growth and support development.

30 Features:

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-Reduces the need for caloric and vitamin supplementation. More calories (22 Cal/f1 oz) than standard term formulas (20 Cal/f1 oz).

-Highly absorbed fat blend, with medium-chain triglycerides (MCT oil) to help meet the special digestive needs of premature infants.

-Higher levels of protein, vitamins and minerals per 100 calories to extend the nutritional support initiated in-hospital.

10 -More calcium and phosphorus for improved bone mineralization.

Ingredients: -D Corn syrup solids, nonfat milk, lactose, whey protein concentrate, soy oil, high-oleic safflower oil, fractionated coconut oil (medium chain triglycerides), coconut oil, potassium citrate, calcium phosphate tribasic, calcium carbonate, ascorbic acid, magnesium chloride, potassium chloride, sodium chloride, taurine, ferrous sulfate, m-inositol, choline chloride, ascorbyl palmitate, L-carnitine, alpha-tocopheryl acetate, zinc sulfate, niacinamide, mixed tocopherols, sodium citrate, calcium pantothenate, cupric sulfate, thiamine chloride hydrochloride, vitamin A palmitate, beta carotene, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, vitamin D3, sodium selenite and cyanocobalamin.

15 Various PUFAs of this invention can be substituted and/or added to the infant formulae described above and to other infant formulae known to those in the art.

#### II. NUTRITIONAL FORMULATIONS

20 niacinamide, mixed tocopherols, sodium citrate, calcium pantothenate, cupric sulfate, thiamine chloride hydrochloride, vitamin A palmitate, beta carotene, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

25 G. Similac Natural Care Low-Iron Human Milk Fortifier Ready To Use, 24 Cal/f1 oz. :

Usage: Designed to be mixed with human milk or to be fed alternatively with human milk to low-birth-weight infants.

30 Patient Conditions:

-For patients on modified diets

-For elderly patients at nutrition risk

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- For patients with involuntary weight loss
- For patients recovering from illness or surgery
- For patients who need a low-residue diet.

5 Ingredients: -D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinate, High-Oleic Safflower Oil, Soy Protein Isolate, Soy Oil, Canola Oil, Potassium Citrate, Calcium Phosphate Tribasic, Sodium Citrate, Magnesium Chloride, Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride, 10 Soy Lecithin, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Gellan Gum, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Vitamin A, Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Sodium Molybdate, Chromium Chloride, Biotin, Potassium Iodide, Sodium Selenate.

B. ENSURE® BARS:

15 Usage: ENSURE BARS are complete, balanced nutrition for supplemental use between or with meals. They provide a delicious, nutrient-rich alternative to other snacks. ENSURE BARS contain <1 g lactose/bar, and Chocolate Fudge Brownie flavor is gluten-free. (Honey Graham Crunch flavor contains 25 gluten.)

Patient Conditions:

- For patients who need extra calories, protein, vitamins and minerals.
- Especially useful for people who do not take in enough calories and nutrients.

- For people who have the ability to chew and swallow
- Not to be used by anyone with a peanut allergy or any type of allergy to nuts.

Ingredients: Honey Graham Crunch -- High-Fructose Corn Syrup, 5 Soy Protein Isolate, Brown Sugar, Honey, Maltodextrin (Corn), Crisp Rice (Milled Rice, Sugar [Sucrose], Salt [Sodium Chloride] and Malt), Oat Bran, Partially Hydrogenated Cottonseed and Soy Oils, Soy Polyaccharide, Glycerine, Whey Protein Concentrate, 10 Polydextrose, Fructose, Calcium Caseinate, Cocoa Powder, Artificial Flavors, Canola Oil, High-Oleic Safflower Oil, Nonfat Dry Milk, Whey Powder, Soy Lecithin and Corn Oil. Manufactured in a facility that processes nuts.

15 Vitamins and Minerals: Calcium Phosphate Tribasic, Potassium Phosphate Dibasic, Magnesium Oxide, Salt (Sodium Chloride), Potassium Chloride, Ascorbic Acid, Ferric Orthophosphate, Alpha-Tocopheryl Acetate, Niacinamide, Zinc Oxide, Calcium Pantothenate, Copper Gluconate, Manganese, Zinc Oxide, Riboflavin, Beta Carotene, Pyridoxine Hydrochloride, Thiamine Mononitrate, Folic Acid, Biotin, Chromium Chloride, Potassium Iodide, Sodium Selenate, Sodium Molybdate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

20 Protein: Honey Graham Crunch - The protein source is a blend of soy protein isolate and milk proteins.

25 Soy protein isolate 74¢

30 Milk proteins 26¢

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**Fat:** Honey Graham Crunch - The fat source is a blend of partially hydrogenated cottonseed and soybean, canola, high oleic safflower, oils, and soy lecithin.

5 Partially hydrogenated cottonseed and soybean oil 76%  
 Canola oil 8%  
 High-oleic safflower oil 8%  
 Corn oil 4%  
 Soy lecithin 4%

10 Carbohydrate: Honey Graham Crunch - The carbohydrate source is a combination of high-fructose corn syrup, brown sugar, maltodextrin, honey, crisp rice, glycerine, soy polysaccharide, and oat bran.

15 High-fructose corn syrup 24%  
 Brown sugar 21%  
 Maltodextrin 12%  
 Honey 11%  
 20 Crisp rice 9%  
 Glycerine 9%  
 Soy Polysaccharide 7%  
 Oat bran 7%

25 C. ENSURE® HIGH PROTEIN:

Usage: ENSURE HIGH PROTEIN is a concentrated, high-protein liquid food designed for people who require additional calories, protein, vitamins, and minerals in their diets. It can be used as an oral nutritional supplement with or between meals or, in appropriate amounts, as a meal replacement. ENSURE HIGH PROTEIN

is lactose- and gluten-free, and is suitable for use by people recovering from general surgery or hip fractures and by patients at risk for pressure ulcers.

5 Patient Conditions:

-For patients who require additional calories, protein, vitamins, and minerals, such as patients recovering from general surgery or hip fractures, patients at risk for pressure ulcers, and patients on low-cholesterol diets

10 Features:

-Low in saturated fat  
 -Contains 6 g of total fat and < 5 mg of cholesterol per serving  
 -Rich, creamy taste  
 -Excellent source of protein, calcium, and other essential vitamins and minerals  
 -For low-cholesterol diets  
 -Lactose-free, easily digested

15 Ingredients:

Vanilla Supreme: -D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinates, High-Oleic Safflower Oil, Soy Protein Isolate, Soy Oil, Canola Oil, Potassium Citrate, Calcium Phosphate Tribasic, Sodium Citrate, Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride, Soy Lecithin, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Gellan Gum, Niacinamide.

30 Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine

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Hydrochloride, Riboflavin, Folic Acid, Sodium Molybdate, Chromium Chloride, Biotin, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

## 5 Protein:

The protein source is a blend of two high-biologic-value proteins: casein and soy.

Sodium and calcium caseinates 85%  
Soy protein isolate 15%

## Fat:

The fat source is a blend of three oils: high-oleic safflower, canola, and soy.

High-oleic safflower oil 40%  
Canola oil 30%  
Soy oil 30%

20 The level of fat in ENSURE HIGH PROTEIN meets American Heart Association (AHA) Guidelines. The 6 grams of fat in ENSURE HIGH PROTEIN represent 24% of the total calories, with 2.6% of the fat being from saturated fatty acids and 7.9% from polyunsaturated fatty acids. These values are within the AHA guidelines of < 30% of total calories from fat, < 10% of the calories from saturated fatty acids, and < 10% of total calories from polyunsaturated fatty acids.

## Carbohydrate:

30 ENSURE HIGH PROTEIN contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla

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supreme, chocolate royal, wild berry, and banana), plus VARI- FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

## 5 Vanilla and other nonchocolate flavors:

Sucrose 60%  
Maltodextrin 40%

## Chocolate:

Sucrose 70%  
Maltodextrin 30%

15 D. ENSURE® LIGHT  
Usage: ENSURE LIGHT is a low-fat liquid food designed for use as an oral nutritional supplement with or between meals. ENSURE LIGHT is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

## Patient Conditions:

25 -For normal-weight or overweight patients who need extra nutrition in a supplement that contains 50% less fat and 20% fewer calories than ENSURE.

-For healthy adults who don't eat right and need extra nutrition.

## 30 Features:

30 ENSURE HIGH PROTEIN contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla

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- Low in fat and saturated fat
- Contains 3 g of total fat per serving and < 5 mg cholesterol
- Rich, creamy taste
- Excellent source of calcium and other essential vitamins and minerals
- For low-cholesterol diets
- Lactose-free, easily digested

## Ingredients:

10 French Vanilla: -D Water, Maltodextrin (Corn), Sugar (Sucrose), Calcium Caseinate, High-Oleic Safflower Oil, Canola Oil, Magnesium Chloride, Sodium Citrate, Potassium Citrate, Potassium Phosphate Dibasic, Magnesium Phosphate

15 Dibasic, Natural and Artificial Flavor, Calcium Phosphate Tribasic, Cellulose Gel, Choline Chloride, Soy Lecithin, Carrageenan, Salt (Sodium Chloride), Ascorbic Acid, Cellulose Gum, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfate, Calcium Pantothenate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Vitamin A Palmitate, Pyridoxine Hydrochloride, Riboflavin, Chromium Chloride, Folic Acid, Sodium Molybdate, Biotin, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

20 Protein: The protein source is calcium caseinate.

130

- The fat source is a blend of two oils: high-oleic safflower and canola.

|   |                          |     |
|---|--------------------------|-----|
| 5 | High-oleic safflower oil | 70% |
|   | Canola oil               | 30% |

## The level of fat in ENSURE LIGHT meets American Heart

Association (AHA) guidelines. The 3 grams of fat in ENSURE LIGHT represent 13.5% of the total calories, with 1.4% of the fat being from saturated fatty acids and 2.6% from polyunsaturated fatty acids. These values are within the AHA guidelines of < 30% of total calories from fat, < 10% of the, calories from saturated fatty acids, and < 10% of total calories from polyunsaturated fatty acids.

## Carbohydrate:

10 ENSURE LIGHT contains a combination of maltodextrin and sucrose. The chocolate flavor contains corn syrup as well. The mild 20 sweetness and flavor variety (French vanilla, chocolate supreme, strawberry swirl), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

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## Vanilla and other nonchocolate flavors:

|    |              |     |
|----|--------------|-----|
| 25 | Vanilla      | 51% |
| 30 | Maltodextrin | 49% |
|    | Chocolate:   |     |

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132

## Ingredients:

Sucrose 47.0%  
 Corn Syrup 26.5%  
 Maltodextrin 26.5%

## 5 Vitamins and Minerals:

An 8-fl.oz serving of ENSURE LIGHT provides at least 25% of the RDIs for 24 key vitamins and minerals.

## 10 Caffeine:

Chocolate flavor contains 2.1 mg caffeine/8 fl. oz.

## E. ENSURE PLUS®

Usage: ENSURE PLUS is a high-calorie, low-residue liquid food for use when extra calories and nutrients, but a normal concentration of protein, are needed. It is designed primarily as an oral nutritional supplement to be used with or between meals or, in appropriate amounts, as a meal replacement. ENSURE PLUS is lactose- and gluten-free. Although it is primarily an oral nutritional supplement, it can be fed by tube.

15 Protein:

The protein source is a blend of two high-biologic-value proteins: casein and soy.

16 Patient Conditions:

-For patients who require extra calories and nutrients, but a normal concentration of protein, in a limited volume

20 -For patients who need to gain or maintain healthy weight

## Features:

-Rich, creamy taste  
 30 -Good source of essential vitamins and minerals

25 Carbohydrate:

The fat source is corn oil.

26 ENSURE PLUS contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, strawberry, coffee, buffer pecan, and eggnog), plus

100%  
 Corn oil

Fat:  
 The fat source is corn oil.

20 Sodium and calcium caseinates 84%  
 Soy protein isolate 16%

Protein:

25

Carbohydrate:

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VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

**5** Vanilla, strawberry, butter pecan, and coffee flavors:

|              |     |                                      |
|--------------|-----|--------------------------------------|
| Corn Syrup   | 39% | Features:                            |
| Maltodextrin | 38% | -For supplemental or total nutrition |
| Sucrose      | 23% | -For oral or tube feeding            |
|              | 10  | -1.5 Cal/mL,                         |
|              |     | -High nitrogen                       |
|              |     | -Calorically dense                   |
|              |     |                                      |
| Corn Syrup   | 36% | Ingredients:                         |
| Maltodextrin | 34% |                                      |
| Sucrose      | 30% |                                      |

Vitamins and Minerals:

An 8-fl-oz serving of ENSURE PLUS provides at least 15% of the RDIs for 25 key Vitamins and minerals.

**20** Caffeine:

Chocolate flavor contains 3.1 mg Caffeine/8 fl oz. Coffee flavor contains a trace amount of caffeine.

**25** F. ENSURE PLUS® HN

Usage: ENSURE PLUS HN is a nutritionally complete high-calorie, high-nitrogen liquid food designed for people with higher calorie and protein needs or limited volume tolerance. It may be used for oral supplementation or for total nutritional support by tube. ENSURE PLUS HN is lactose- and gluten-free.

**G. ENSURE® POWDER:**

30

Usage: ENSURE POWDER (reconstituted with water) is a low-residue

Patient Conditions:

- For patients with increased calorie and protein needs, such as following surgery or injury.
- 5** -For patients with limited volume tolerance and early satiety.

|              |     |                                      |
|--------------|-----|--------------------------------------|
| Corn Syrup   | 39% | Features:                            |
| Maltodextrin | 38% | -For supplemental or total nutrition |
| Sucrose      | 23% | -For oral or tube feeding            |
|              | 10  | -1.5 Cal/mL,                         |

Chocolate and eggnog flavors:

|              |     |                                      |
|--------------|-----|--------------------------------------|
| Corn Syrup   | 36% | Features:                            |
| Maltodextrin | 34% | -For supplemental or total nutrition |
| Sucrose      | 30% | -For oral or tube feeding            |
|              | 15  | -1.5 Cal/mL,                         |

Vanilla: -D Water, Maltodextrin (Corn), Sodium and Calcium Caseinates, Corn Oil, Sugar (Sucrose), Soy Protein Isolate, Magnesium Chloride, Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial Flavor, Sodium Citrate, Choline Chloride, Ascorbic Acid, Taurine, L-Carnitine, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Carrageenan, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium Molybdate, Potassium Iodide, Sodium Selenite, Phylloquinone, Cyanocobalamin and Vitamin D3.

**20** G. ENSURE® POWDER:

Usage: ENSURE POWDER (reconstituted with water) is a low-residue

**G. ENSURE® POWDER:**

30

Usage: ENSURE POWDER (reconstituted with water) is a low-residue

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Liquid food designed primarily as an oral nutritional supplement to be used with or between meals. ENSURE POWDER is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

5 Patient Conditions:

- For patients on modified diets
- For elderly patients at nutrition risk
- For patients recovering from illness/surgery
- 10 -For patients who need a low-residue diet

Features:

- Convenient, easy to mix
- Low in saturated fat
- 15 -Contains 9 g of total fat and < 5 mg of cholesterol per serving
- High in vitamins and minerals
- For low-cholesterol diets
- Lactose-free, easily digested
- 20 Ingredients: -D Corn Syrup, Maltodextrin (Corn), Sugar (Sucrose), Corn Oil, Sodium and Calcium Caseinates, Soy Protein Isolate, Artificial Flavor, Potassium Citrate, Magnesium Chloride, Sodium Citrate, Calcium Phosphate Tribasic, Potassium Chloride, Soy Lecithin, Ascorbic Acid, Choline Chloride, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Thiamine Chloride Hydrochloride, Cupric Sulfate, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Sodium Molybdate, Chromium Chloride, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.
- 25 H. ENSURE<sup>®</sup> PUDDING
- 30 Usage: ENSURE PUDDING is a nutrient-dense supplement providing balanced nutrition in a nonliquid form to be used with or between meals. It is appropriate for consistency-modified diets

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Protein:  
The protein source is a blend of two high-biologic-value proteins: casein and soy.

5 Sodium and calcium caseinates 84%

Soy protein isolate 16%

Features:  
Fat:  
10 The fat source is corn oil.

Corn oil 100%

Carbohydrate:

15 ENSURE POWDER contains a combination of corn syrup, maltodextrin, and sucrose. The mild sweetness of ENSURE POWDER, plus VARI-FLAVORS<sup>®</sup> Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, helps to prevent flavor fatigue and aid in patient compliance.

20

Ingredients: -D Corn Syrup, Maltodextrin (Corn), Sugar (Sucrose), Corn Oil, Sodium and Calcium Caseinates, Soy Protein Isolate, Artificial Flavor, Potassium Citrate, Magnesium Chloride, Sodium Citrate, Calcium Phosphate Tribasic, Potassium Chloride, Soy Lecithin, Ascorbic Acid, Choline Chloride, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Thiamine Chloride Hydrochloride, Cupric Sulfate, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Sodium Molybdate, Chromium Chloride, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

H. ENSURE<sup>®</sup> PUDDING

Usage: ENSURE PUDDING is a nutrient-dense supplement providing balanced nutrition in a nonliquid form to be used with or between meals. It is appropriate for consistency-modified diets

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(e.g., soft, pureed, or full liquid) or for people with swallowing impairments. ENSURE PUDDING is gluten-free.

Patient Conditions:

- 5 -For patients on consistency-modified diets (e.g., soft, pureed, or full liquid)
- For patients with swallowing impairments

Features:

- 10 -Rich and creamy, good taste
- Good source of essential vitamins and minerals
- Convenient-needs no refrigeration
- Gluten-free

15 Nutrient Profile per 5 oz: Calories 250, Protein 10.9%, Total Fat 34.9%, Carbohydrate 54.2%

Ingredients:

Vanilla: -D Nonfat Milk, Water, Sugar (Sucrose), Partially Hydrogenated Soybean Oil, Modified Food Starch, Magnesium Sulfate, Sodium Stearoyl Lactylate, Sodium Phosphate Dibasic, Artificial Flavor, Ascorbic Acid, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Choline Chloride, Niacinamide, Manganese Sulfate, Calcium Pantothenate, FD&C Yellow #5, Potassium Citrate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, FD&C Yellow #6, Folic Acid, Biotin, Phylloquinone, Vitamin D3 and Cyanocobalamin.

30 Protein:

The protein source is nonfat milk.

Nonfat milk  
100%

5 Fat:  
-For patients with swallowing impairments

Hydrogenated soybean oil  
100%

10 Carbohydrate:

ENSURE PUDDING contains a combination of sucrose and modified food starch. The mild sweetness and flavor variety (vanilla, chocolate, butterscotch, and tapioca) help prevent flavor fatigue. The product contains 9.2 grams of lactose per serving.

Vanilla and other nonchocolate flavors:

20 Sucrose  
Lactose  
Modified food starch  
Chocolate:  
25 Sucrose  
Lactose  
Modified food starch  
16%

30 I. ENSURE® WITH FIBER:

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The protein source is nonfat milk.

Nonfat milk  
100%

5 Fat:  
-For patients with swallowing impairments

Hydrogenated soybean oil  
100%

10 Carbohydrate:

ENSURE PUDDING contains a combination of sucrose and modified food starch. The mild sweetness and flavor variety (vanilla, chocolate, butterscotch, and tapioca) help prevent flavor fatigue. The product contains 9.2 grams of lactose per serving.

Vanilla and other nonchocolate flavors:

20 Sucrose  
Lactose  
Modified food starch  
Chocolate:  
25 Sucrose  
Lactose  
Modified food starch  
16%

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Usage: ENSURE WITH FIBER is a fiber-containing, nutritionally complete liquid food designed for people who can benefit from increased dietary fiber and nutrients. ENSURE WITH FIBER is suitable for people who do not require a low-residue diet. It can be fed orally or by tube, and can be used as a nutritional supplement to a regular diet or, in appropriate amounts, as a meal replacement. ENSURE WITH FIBER is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

10

## Patient Conditions:

- For patients who can benefit from increased dietary fiber and nutrients

## 15 Features:

- New advanced formula-low in saturated fat, higher in vitamins and minerals
- Contains 6 g of total fat and < 5 mg of cholesterol per serving
- Rich, creamy taste

20 -Good source of fiber

-Excellent source of essential vitamins and minerals

-For low-cholesterol diets

-Lactose- and gluten-free

## 25 Ingredients:

Vanilla: -D Water; Maltodextrin (Corn), Sugar (Sucrose), Sodium and Calcium Caseinates, Oat Fiber, High-Oleic Safflower Oil, Canola Oil, Soy Protein Isolate, Corn Oil, Soy Fiber, Calcium Phosphate Tribasic, Magnesium Chloride, Potassium Citrate, 30 Cellulose Gel, Soy Lecithin, Potassium Phosphate Dibasic, Sodium Citrate, Natural and Artificial Flavors, Choline Chloride,

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Magnesium Phosphate, Ascorbic Acid, Cellulose Gum, Potassium Chloride, Carrageenan, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfite, Calcium Pantothenate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Chromium Chloride, Biotin, Sodium Molybdate, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

## 10 Protein:

The protein source is a blend of two high-biologic-value proteins-casein and soy.

Sodium and calcium caseinates

80%

15 Soy protein isolate

20%

## Fat:

The fat source is a blend of three oils: high-oleic safflower, canola, and corn.

20

High-oleic safflower oil

Canola oil

Corn oil

25

The level of fat in ENSURE WITH FIBER meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE WITH FIBER represent 22% of the total calories, with 2.01% of the fat being from saturated fatty acids and 6.7% from polyunsaturated fatty acids. These values are within the AHA guidelines of  $\leq 30\%$  of total calories from fat, < 10% of the

30

calories from saturated fatty acids, and  $\leq 10\%$  of total calories from polyunsaturated fatty acids.

**Carbohydrate:**

5 ENSURE WITH FIBER contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, and butter pecan), plus VARI-FLAVORS<sup>®</sup> Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

10 Vanilla and other nonchocolate flavors:

|              |     |
|--------------|-----|
| Maltodextrin | 66% |
| Sucrose      | 25% |
| Oat Fiber    | 7%  |
| Soy Fiber    | 2%  |

**Chocolate:**

|              |     |
|--------------|-----|
| Maltodextrin | 55% |
| Sucrose      | 36% |
| Oat Fiber    | 7%  |
| Soy Fiber    | 2%  |
| Fiber:       | 25  |

The fiber blend used in ENSURE WITH FIBER consists of oat fiber and soy polysaccharide. This blend results in approximately 4 grams of total dietary fiber per 8-fl. oz can. The ratio of insoluble to soluble fiber is 95:5.

30

The various nutritional supplements described above and known to others of skill in the art can be substituted and/or supplemented with the PUFAs produced in accordance with the present invention.

5 **J. Oxepa<sup>™</sup> Nutritional Product**  
Oxepa is a low-carbohydrate, calorically dense, enteral nutritional product designed for the dietary management of patients with or at risk for ARDS. It has a unique combination of ingredients, including a patented oil blend containing eicosapentaenoic acid (EPA from fish oil),  $\gamma$ -linolenic acid (GLA from borage oil), and elevated antioxidant levels.

15 **Caloric Distribution:**  
Caloric density is high at 1.5 Cal/mL (355 Cal/8 fl.oz), to minimize the volume required to meet energy needs. The distribution of calories in Oxepa is shown in Table IV.

|                  | 20   | Table IV. Caloric Distribution of Oxepa |           |          |
|------------------|------|---|-----------|----------|
|                  |      | per 8 fl. oz.                           | per liter | % of Cal |
| Calories         |      | 355                                     | 1,500     | ---      |
| Fat (g)          |      | 22.2                                    | 93.7      | 55.2     |
| Carbohydrate (g) | 25   |   | 105.5     | 28.1     |
| Protein (g)      | 14.8 | 62.5                                    | 16.7      |          |
| Water (g)        | 186  | 785                                     | ---       |          |

Fat:

-Oxepa contains 22.2 g of fat per 8-fl. oz serving (93.7 g/L).  
-The fat source is an oil blend of 31.8% canola oil, 25% medium-chain triglycerides (MCTs), 20% borage oil, 20% fish oil, and

30

3.2 % soy lecithin. The typical fatty acid profile of Oxepa is shown in Table V.

-Oxepa provides a balanced amount of polyunsaturated, 5 monounsaturated, and saturated fatty acids, as shown in Table VI.

-Medium-chain triglycerides (MCTs) -- 25% of the fat blend -- aid gastric emptying because they are absorbed by the intestinal 10 tract without emulsification by bile acids.

The various fatty acid components of Oxepa™ nutritional product can be substituted and/or supplemented with the PUFAs produced in accordance with this invention.

15

|    |                      | % Total | g/8 fl oz* | g/L*  |
|----|----------------------|---------|------------|-------|
|    | Fatty Acids          |         |            |       |
| 5  |                      |         |            |       |
|    | Caproic (6:0)        | 0.2     | 0.04       | 0.18  |
|    | Caprylic (8:0)       | 14.69   | 3.1        | 13.07 |
| 10 | Capric (10:0)        | 11.06   | 2.33       | 9.87  |
|    | Palmitic (16:0)      | 5.59    | 1.18       | 4.98  |
|    | Palmitoleic          | 1.82    | 0.38       | 1.62  |
|    | Stearic              | 1.94    | 0.39       | 1.64  |
| 15 | Oleic                | 24.44   | 5.16       | 21.75 |
|    | Linoleic             | 16.28   | 3.44       | 14.49 |
|    | α-Linolenic          | 3.47    | 0.73       | 3.09  |
|    | γ-Linolenic          | 4.82    | 1.02       | 4.29  |
|    | Eicosapentaenoic     | 5.11    | 1.08       | 4.55  |
|    | n-3-Docosapentaenoic | 0.55    | 0.12       | 0.49  |
| 20 | 27                   | 0.48    | 2.02       |       |
|    | Docosahexaenoic      | 2.27    |            |       |
|    | Others               | 7.55    | 1.52       | 6.72  |

25 Fatty acids equal approximately 95% of total fat.

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Table VI. Fat Profile of Oxepa.

|                              |                              |
|------------------------------|------------------------------|
| % of total calories from fat | 55.2                         |
| Polyunsaturated fatty acids  | 31.44 g/L                    |
| Monounsaturated fatty acids  | 25.53 g/L                    |
| Saturated fatty acids        | 32.38 g/L                    |
| n-6 to n-3 ratio             | 1.75:1                       |
| Cholesterol                  | 9.49 mg/8 fl oz<br>40.1 mg/L |

## Carbohydrate:

10 -The carbohydrate content is 25.0 g per 8-fl-oz serving (105.5 g/L).

11 -The carbohydrate sources are 45% maltodextrin (a complex carbohydrate) and 55% sucrose (a simple sugar), both of which are readily digested and absorbed.

12 -The high-fat and low-carbohydrate content of Oxepa is designed to minimize carbon dioxide (CO<sub>2</sub>) production. High CO<sub>2</sub> levels can complicate weaning in ventilator-dependent patients. The low level of carbohydrate also may be useful for those patients who have developed stress-induced

20 hyperglycemia.

-Oxepa is lactose-free.

carbohydrate level in Oxepa is also sufficient to minimize gluconeogenesis, if energy needs are being met.

## Protein:

5 -Oxepa contains 14.8 g of protein per 8-fl-oz serving (62.5 g/L).

6 -The total calorie/nitrogen ratio (150:1) meets the need of stressed patients.

7 -Oxepa provides enough protein to promote anabolism and the maintenance of lean body mass without precipitating respiratory problems. High protein intakes are a concern in patients with respiratory insufficiency. Although

8 protein has little effect on CO<sub>2</sub> production, a high protein diet will increase ventilatory drive.

10 -The protein sources of Oxepa are 86.8% sodium caseinate and 13.2% calcium caseinate.

11 -The amino acid profile of the protein system in Oxepa meets or surpasses the standard for high quality protein set by the National Academy of Sciences.

20

\* Oxepa is gluten-free.

Dietary carbohydrate, the amino acids from protein, and the glycerol moiety of fats can be converted to glucose within the body. Throughout this process, the carbohydrate requirements of glucose-dependent tissues (such as the central nervous system and red blood cells) are met. However, a diet free of carbohydrates can lead to ketosis, excessive catabolism of tissue protein, and loss of fluid and electrolytes. These effects can be prevented by daily ingestion of 50 to 100 g of digestible carbohydrate, if caloric intake is adequate. The

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20

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Default settings for the analysis programs**GCG Programs**FastA Search

## Default parameters:

range of interest Begin=1 END=last protein or nucleic acid

search set all of SwissProt (protein) or GenEMBL (nucleic acid)

word size = (2) for protein = (6) for nucleic acid

Expected scores lists scores until E( ) value reaches 2.0

Pileup

## Default parameters:

range of interest Begin=1 END=last nucleic acid

search set all of GenEMBL

word size wordsz= (2)

Expected scores lists scores until E( ) value reaches 2.0

Sequencher Program

## Default parameters:

gap creation penalty

gap extension penalty

plot figure

one page plot density = 2.7

BLAST 2 Command Line Arguments

number of best scores to show  
 number of best alignments to show  
 [Real] default = 10.0  
 -m Alignment view options:  
 0 = pairwise,  
 1 = master-slave showing identities,  
 2 = master-slave, no identities,  
 3 = flat master-slave, show identities,  
 4 = flat master-slave, no identities,  
 5 = master-slave, no identities  
 6 = flat master-slave, no identities and blunt ends,  
 [Integer]  
 default = 0

(DUST with blastn, SEG with others) [T/F]  
 default = T

-G Cost to open a gap (zero invokes default behavior) [Integer]  
 default = 0

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Automatic Assembly

Dirty data algorithm = slower contig assembly but more rigorous comparisons between the sequences

minimum match = 85%

minimum overlap = 20

BLAST 2 (blastp, tblastn)

Default parameters:  
 V=50  
 B=50  
 E=10  
 Lambda=329  
 K=0.140  
 R=0.427

blast n

Default parameters:  
 V=100  
 B=250  
 E=10  
 Lambda=1.37  
 K=0.171  
 R=1.31

blast n

BLAST 2 Command Line Arguments

-V Hits  
 -b Alignments  
 -e Expectation value (E)  
 -m Alignment view options:  
 0 = pairwise,  
 1 = master-slave showing identities,  
 2 = master-slave, no identities,  
 3 = flat master-slave, show identities,  
 4 = flat master-slave, no identities,  
 5 = master-slave, no identities  
 6 = flat master-slave, no identities and blunt ends,  
 [Integer]  
 default = 0

Sequencher Program

## Default parameters:

gap weight = 5

gap length weight = 12

plot figure

one page plot density = 2.7

Sequencher Program

## Default parameters:

(DUST with blastn, SEG with others) [T/F]  
 default = T

-G Cost to open a gap (zero invokes default behavior) [Integer]  
 default = 0

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```

-E Cost to extend a gap (zero invokes default behavior) [Integer]
  default = 0

-X X dropoff value for gapped alignment (in bits) (zero invokes
  default behavior) [Integer]
  default = 0

-I Show GI's in defines [T/F]
  default = F

-q Penalty for a nucleotide mismatch (blastn only) [Integer]
  default = -3

-r Reward for a nucleotide match (blastn only) [Integer]
  default = 1

-f Threshold for extending hits default if zero [Integer]
  default = 0

-g Perform gapped alignment (not available with tblastx) [T/F]
  default = T

-q Query Genetic code to use [Integer]
  default = 1

-D DB Genetic code [Integer] (for tblast [mx] only)
  default = 1

-J Believe the query define [T/F]
  default = F

-M Matrix default = BLOSUM62 [String]
  default if zero [Integer]

-W Word size default = 0
  default if zero [Integer]

-z Effective length of the database (use zero for the real size)
  [Integer]
  default = 0

-a Number of processors to use [Integer]
  default = site configurable (SeqServer.conf)
  default = 0

```

Allowed and default values for gap open/gap extension cost (-G/-E) parameters:

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CLAIMS:

1. An isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence comprising SEQ ID NO:5 (Figure 54).  
5 2. The isolated nucleotide sequence of claim 1 wherein said sequence comprises SEQ ID NO:5.

10 3. The isolated nucleotide sequence of claims 1 or 2 wherein said sequence encodes a functionally active elongase which utilizes a polyunsaturated fatty acid as a substrate.

15 4. The nucleotide sequence of claim 1 wherein said sequence is derived from a mammal.

20 5. The nucleotide sequence of claim 4 wherein said sequence is derived from a mouse.

6. A purified protein encoded by said nucleotide sequence of claims 1 or 2.

25 7. A purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of said purified protein of claim 6.

8. A method of producing an elongase enzyme comprising the steps of:

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- a) isolating a nucleotide sequence comprising SEQ ID NO:5 (Figure 54) or SEQ ID NO:6 (Figure 58);
- b) constructing a vector comprising: 1) said isolated nucleotide sequence operably linked to ii) a promoter;
- c) introducing said vector into a host cell under time and conditions sufficient for expression of said elongase enzyme.

9. The method of claim 8 wherein said host cell is selected from the group consisting of a eukaryotic cell or a prokaryotic cell.

10 10. The method of claim 9 wherein said prokaryotic cell is selected from the group consisting of *E. coli*, *cyanobacteria*, and *B. subtilis*.

15 11. The method of claim 9 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.

20 12. The method of claim 11 wherein said fungal cell is selected from the group consisting of *Saccharomyces spp.*, *Candida spp.*, *Lipomyces starkey*, *Yarrowia spp.*, *Kluyveromyces spp.*, *Hansenula spp.*, *Aspergillus spp.*, *Penicillium spp.*, *Neurospora spp.*, *Trichoderma spp.* and *Pichia spp.*.

25 13. The method of claim 12 wherein said fungal cell is a yeast cell selected from the group consisting of *Saccharomyces spp.*, *Candida spp.*, *Hansenula spp.* and *Pichia spp.*

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14. The method of claim 13 wherein said yeast cell is Saccharomyces cerevisiae.

5 15. A vector comprising: a) a nucleotide sequence comprising SEQ ID NO:5 (Figure 54) operably linked to b) a promoter.

16. A host cell comprising said vector of claim 15.

10 17. The host cell of claim 16, wherein said host cell is selected from the group consisting of a eukaryotic cell or a prokaryotic cell.

15 18. The host cell of claim 17 wherein said prokaryotic cell is selected from the group consisting of E. coli, Cyanobacteria, and B. subtilis.

19. The host cell of claim 17 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.

20 20. The host cell of claim 19 wherein said cell is selected from the group consisting of Saccharomyces spp., Candida spp., Libomyces starkeyi, Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. and Pichia spp..

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21. The host cell of claim 20 wherein said fungal cell is a yeast cell selected from the group consisting of Saccharomyces spp., Candida spp., Hansenula spp. and Pichia spp..

5 22. The host cell of claim 21 wherein said host cell is Saccharomyces cerevisiae.

23. A plant cell, plant or plant tissue comprising said vector of claim 15, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid by said plant cell, plant or plant tissue.

10 15 24. The plant cell, plant or plant tissue of claim 23 wherein said polyunsaturated fatty acid is selected from the group consisting of AA, ADA, GLA and STA.

25 26. One or more plant oils or acids expressed by said plant cell, plant or plant tissue of claim 23.

20 25 27. A transgenic plant comprising said vector of claim 15, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid in seeds of said transgenic plant.

25 30 SEQ ID NO:5 (Figure 54).

28. A fluid produced by said transgenic, non-human mammal of claim 27 wherein said fluid comprises a detectable level of at least one elongase or products thereof.

5 29. A method for producing a polyunsaturated fatty acid comprising the steps of:

a) isolating a nucleotide sequence comprising SEQ ID NO:5 (Figure 54);

10 b) constructing a vector comprising said isolated nucleotide sequence;

c) introducing said vector into a host cell under time and conditions sufficient for expression of an elongase enzyme encoded by said isolated nucleotide sequence; and

15 d) exposing said expressed elongase enzyme to a substrate polyunsaturated fatty acid in order to convert said substrate to a product polyunsaturated fatty acid.

20 30. The method according to claim 29, wherein said substrate polyunsaturated fatty acid is selected from the group consisting of GLA, STA, AA, ADA and ALA, and said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, ADA,  $\omega$ 6-docosapentaenoic acid and STA, respectively.

25 31. The method according to claim 29 further comprising the step of exposing said expressed elongase enzyme to at least one desaturase in order to convert said product polyunsaturated fatty acid to another polyunsaturated fatty acid.

32. The method according to claim 31 wherein said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, ADA and  $\omega$ 6-docosapentaenoic acid, said another polyunsaturated fatty acid is selected from the group consisting of AA, EPA,  $\omega$ 6-docosapentaenoic acid and docosahexaenoic acid respectively, and said at least one desaturase is  $\Delta$ 5-desaturase with respect to production of AA or EPA, and  $\Delta$ 4-desaturase with respect to production of  $\omega$ 6-docosapentaenoic acid, and  $\Delta$ 19-desaturase with respect to production of docosahexaenoic acid.

5 33. The method of claim 32 further comprising the step of exposing said another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert said another polyunsaturated fatty acid to a final polyunsaturated fatty acid.

10 34. The method of claim 33 wherein said final polyunsaturated fatty acid is selected from the group consisting of AA,  $\omega$ 3-docosapentaenoic acid and docosahexaenoic acid.

15 35. A nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 29, said another polyunsaturated fatty acid produced according to the method

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of claim 31, and said final polyunsaturated fatty acid produced according to the method of claim 33.

36. The nutritional composition of claim 35 wherein said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, ADA,  $\omega$ 6-docosapentaenoic acid and STA.

37. The nutritional composition of claim 35 wherein said another polyunsaturated fatty acid is selected from the group consisting of AA, EPA,  $\omega$ 6-docosapentaenoic acid and docosahexaenoic acid.

38. The nutritional composition of claim 35 wherein said final polyunsaturated fatty acid is selected from the group consisting of ADA,  $\omega$ 3-docosapentaenoic acid and docosahexaenoic acid.

39. The nutritional composition of claim 35 wherein said nutritional composition is selected from the group consisting of an infant formula, a dietary supplement and a dietary substitute.

40. A pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 29, said another polyunsaturated fatty acid produced according to the method of claim 31, and said final polyunsaturated fatty acid

produced according to the method of claim 33 and 2) a pharmaceutically acceptable carrier.

41. An animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 29, said another polyunsaturated fatty acid produced according to the method of claim 31 and said final polyunsaturated fatty acid produced according to the method of claim 33.

42. The animal feed of claim 41 wherein said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, ADA,  $\omega$ 6-docosapentaenoic acid and STA.

43. The animal feed of claim 41 wherein said another polyunsaturated fatty acid is selected from the group consisting of AA, EPA,  $\omega$ 6-docosapentaenoic acid and docosahexaenoic acid.

44. The animal feed of claim 41 wherein said final polyunsaturated fatty acid is selected from the group consisting of ADA,  $\omega$ 3-EPA,  $\omega$ 6-docosapentaenoic acid and docosahexaenoic acid.

45. A cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 29, said another polyunsaturated fatty acid produced according to the method of claim 31, and said final polyunsaturated fatty acid of claim 29, said another polyunsaturated fatty acid

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produced according to the method of claim 33 and 2) a pharmaceutically acceptable carrier.

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produced according to the method of claim 31 and said final polyunsaturated fatty acid produced according to the method of claim 33.

5 46. A method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to said patient said nutritional composition of claim 35 in an amount sufficient to effect said prevention or treatment.

10 47. An isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence comprising SEQ ID NO:6 (Figure 58).

15 48. The isolated nucleotide sequence of claim 47 wherein said sequence comprises SEQ ID NO:6.

20 49. A purified protein encoded by said nucleotide sequence of claims 47 or 48.

25 50. An isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence comprising SEQ ID NO:7 (Figure 72).

30 51. The isolated nucleotide sequence of claim 50 wherein said sequence comprises SEQ ID NO:7.

52. The isolated nucleotide sequence of claims 50 or 51, wherein said sequence encodes a functionally active elongase which utilizes a polyunsaturated fatty acid as a substrate.

53. The nucleotide sequence of claim 50 wherein said sequence is derived from *Thraustochytrium aureum*.

5 54. A purified protein encoded by said nucleotide sequence of claims 50 or 51.

10 55. A purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of said purified protein of claim 54.

15 56. A method of producing an elongase enzyme comprising the steps of:

- a) isolating a nucleotide sequence comprising SEQ ID NO:7 (Figure 72);
- b) constructing a vector comprising: i) said isolated nucleotide sequence operably linked to ii) a promoter;
- c) introducing said vector into a host cell under time and conditions sufficient for expression of said elongase enzyme.

20 57. The method of claim 56 wherein said host cell is selected from the group consisting of a eukaryotic cell or a prokaryotic cell.

25 58. The method of claim 57 wherein said prokaryotic cell is selected from the group consisting of *E. coli*, *cyanobacteria*, and *B. subtilis*.

30 59. The method of claim 57 wherein said eukaryotic

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cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.

60. The method of claim 59 wherein said fungal cell is selected from the group consisting of Saccharomyces spp., Candida spp., Lipomyces starkey, Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. and Pichia spp.

61. The method of claim 59 wherein said fungal cell is a yeast cell selected from the group consisting of Saccharomyces spp., Candida spp., Hansenula spp. and Pichia spp.

62. The method of claim 61 wherein said yeast cell is Saccharomyces cerevisiae.

63. A vector comprising: a) a nucleotide sequence comprising SEQ ID NO:7 (Figure 72) operably linked to b) a promoter.

64. A host cell comprising said vector of claim 63.

65. The host cell of claim 64, wherein said host cell is selected from the group consisting of a eukaryotic cell or a prokaryotic cell.

66. The host cell of claim 65 wherein said prokaryotic cell is selected from the group consisting of E. coli, Cyanobacteria, and B. subtilis.

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67. The host cell of claim 65 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.

68. The host cell of claim 67 wherein said fungal cell is selected from the group consisting of Saccharomyces spp., Candida spp., Lipomyces starkey, Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. and Pichia spp.

69. The host cell of claim 68 wherein said fungal cell is a yeast cell selected from the group consisting of Saccharomyces spp., Candida spp., Hansenula spp. and Pichia spp.

70. The host cell of claim 69 wherein said host cell is Saccharomyces cerevisiae.

71. A plant cell, plant or plant tissue comprising said vector of claim 63, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid by said plant cell, plant or plant tissue.

72. The plant cell, plant or plant tissue of claim 71 wherein said polyunsaturated fatty acid is selected from the group consisting of AA, ADA, GLA and STA.

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73. One or more plant oils or acids expressed by said plant cell, plant or plant tissue of claim 72.

5 74. A transgenic plant comprising said vector of claim 63, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid in seeds of said transgenic plant.

10 75. A transgenic, non-human mammal whose genome comprises a DNA sequence encoding an elongase, operably linked to a promoter, wherein said DNA sequence comprises SEQ ID NO:7 (Figure 72).

15 76. A fluid produced by said transgenic, non-human mammal of claim 75 wherein said fluid comprises a detectable level of at least one elongase or products thereof.

20 77. A method for producing a polyunsaturated fatty acid comprising the steps of:  
a) isolating a nucleotide sequence comprising SEQ ID NO:7 (Figure 72);  
b) constructing a vector comprising said isolated nucleotide sequence;  
c) introducing said vector into a host cell under time and conditions sufficient for expression of an elongase enzyme encoded by said isolated nucleotide sequence; and  
25 d) exposing said expressed elongase enzyme to a substrate polyunsaturated fatty acid in order to convert said substrate to a product polyunsaturated fatty acid.

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78. The method according to claim 77, wherein said substrate polyunsaturated fatty acid is selected from the group consisting of GLA, STA, AA, ADA and ALA, and said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, ADA,  $\omega$ 6-docosapentaenoic acid and STA, respectively.

5 79. The method according to claim 77 further comprising the step of exposing said expressed elongase enzyme to at least one desaturase in order to convert said product polyunsaturated fatty acid to another polyunsaturated fatty acid.

10 80. The method according to claim 79 wherein said product polyunsaturated fatty acid is selected from the group consisting of of DGLA, 20:4n-3, ADA and  $\omega$ 6-docosapentaenoic acid, said another polyunsaturated fatty acid is selected from the group consisting of AA, EPA,  $\omega$ 6-docosapentaenoic acid and docosahexanoic acid respectively, and said at least one desaturase is  $\Delta$ 5-desaturase with respect to production of AA or EPA, and  $\Delta$ 4-desaturase with respect to production of  $\omega$ 6-docosapentaenoic acid, and  $\Delta$ 9-desaturase with respect to production of docosahexanoic acid.

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desaturase in order to convert said another polyunsaturated fatty acid to a final polyunsaturated fatty acid.

82. The method of claim 81 wherein said final polyunsaturated fatty acid is selected from the group consisting of ADA,  $\omega$ 3-docosapentaenoic acid and docosahexaenoic acid.

83. A nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 77, said another polyunsaturated fatty acid produced according to the method of claim 79, and said final polyunsaturated fatty acid produced according to the method of claim 81 and 2) a

produced according to the method of claim 81.

84. The nutritional composition of claim 83 wherein said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, ADA,  $\omega$ 6-docosapentaenoic acid and STA.

85. The nutritional composition of claim 83 wherein said another polyunsaturated fatty acid is selected from the group consisting of AA, EPA,  $\omega$ 6-docosapentaenoic acid and docosahexaenoic acid.

86. The nutritional composition of claim 83 wherein said final polyunsaturated fatty acid is selected from the group consisting of ADA,  $\omega$ 3-docosapentaenoic acid and docosahexaenoic acid.

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87. The nutritional composition of claim 83 wherein said nutritional composition is selected from the group consisting of an infant formula, a dietary supplement and a dietary substitute.

88. A pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 77, said another polyunsaturated fatty acid produced according to the method of claim 79, and said final polyunsaturated fatty acid produced according to the method of claim 81 and 2) a pharmaceutically acceptable carrier.

89. An animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 77, said another polyunsaturated fatty acid produced according to the method of claim 79 and said final polyunsaturated fatty acid produced according to the method of claim 81.

90. The animal feed of claim 89 wherein said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, ADA,  $\omega$ 6-docosapentaenoic acid and STA.

91. The animal feed of claim 89 wherein said another

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polyunsaturated fatty acid is selected from the group consisting of AA, EPA,  $\omega 6$ -docosapentaenoic acid and docosahexaenoic acid.

5 92. The animal feed of claim 89 wherein said final  
polyunsaturated fatty acid is selected from the group  
consisting of ADA,  $\omega_3$ -docosapentaenoic acid and  
docosahexaenoic acid.

93. A cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 77, said another polyunsaturated fatty acid produced according to the method of claim 79 and said final polyunsaturated fatty acid produced according to the method of claim 81.

94. A method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to said patient said nutritional composition of claim 83 in an amount sufficient to effect said prevention or treatment

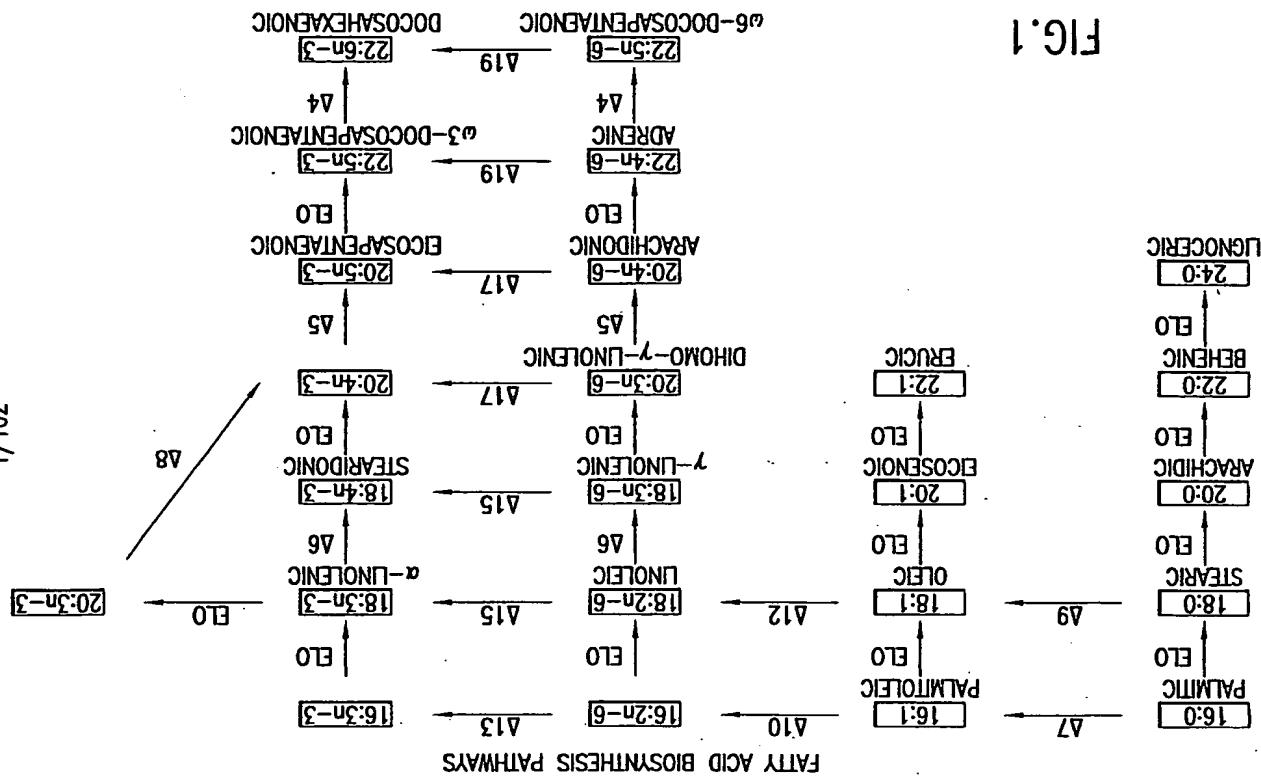


FIG. 1

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Gap Weight: 6      Average Match: 2.912  
 Length weight: 4      Average Mismatch: -2.003  
 Quality: 50      Length: 84  
 Ratio: 0.625      Gaps: 4  
 Percent Similarity: 43.038      Percent Identity: 29.114

Match display thresholds for the alignment(s):

| = IDENTITY  
 : = 2  
 . = 1

jojobakcs x EL02      June 4, 1998 08:23 ..

jojobakcs 24 ATLPNFKSSINLHAKKL.GYHYLISNALFLVFLPLGLASAHLLSSFAHD 72  
 EL02 66 STLPVLYATAYYVIFGRFLLSKS.KPF.KLNGLFQLHNVLTSLS 112

jojobakcs 73 LSLFDLRRNLLPVWCSFLFVLLATLHFTRP 106  
 EL02 113 LTLL.LMVEQVPTIVQHGLYFAICNIGANTQP 145

FIG.2

FIG.3

112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134  
 TCC CTC CTC CTC CTC CTC AGC ATG GTC GAG CAG CTC GTC CTC ACC ATC GTC CAC GAG CTC TAC TAC TAC  
 S L T L L L M V E D Q L V P I I V Q H G L Y F  
 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111  
 CTC TCC AGC TCC AGC CTC ACC TCC AGC GTC CTC TAC GTC CAC ACC CTC ACC TCC CTC  
 L S K S K P F K L N G L F D Q L H N L V L T S L  
 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88  
 TCC ACC CTC CTC CTC GTC CTC ACC GTC ACC TAC GTC ATC ATC TCC GTC CTC TCC CTC  
 S T L P P V L Y A I T A Y V I I F G R F L  
 <-- RO339

S. cerevisiae EL02 (AA66-145) with M. alpina codon bias

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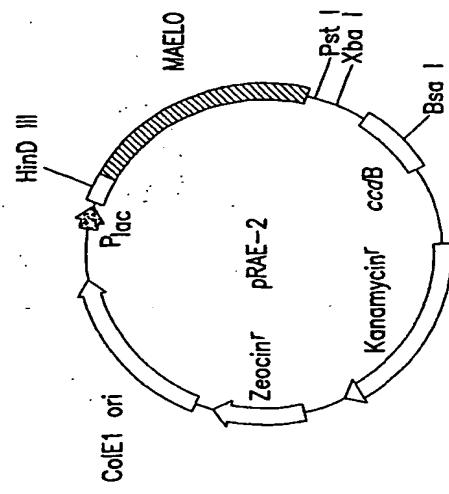


FIG. 4A

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FIG. 5

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1 ATGGCCGCG CAATCTGGA CAAGGTCAAC TTGGGCAATTG ATAGGCCCT  
 51 CGGATAAAG CTGACACCT ACTTTCTCA GGCCTATGAA CTGGTACCG  
 101 GAAAGTCCAT CGACTCCCTC GTCTTCAGG AGGGTCAAC GCCTCTCG  
 151 ACCAGAGAG AGGTGGCAT GTGGGACTATC ACTTACTTCG TCGCATCT  
 201 TGGTGGTCG CAGATCATGA AGAGCAGGA CGCCTCAAG CTAAGCCCC  
 251 TCTTCATCTT CCAAACTTC CTTCGAGA TCGGTCGG ATGGCTGG  
 301 CTCCTGTCAGA TCGGAAACCTT GTTCCCAATC CTGGCAGAA AGCGACTTT  
 351 CTAGGCCATC TGCGACGAG GTGCTGGAC CGACGGCTC GAGCTCTCT  
 401 ACTACCTCAA CTAACTGGTC AAGTGGTGG AGTGGCGGA CACCGCTTT  
 451 TTGGTCCCTCA AGAAGAGGCC TCTTGAAGTTC CTGGACTACT TCCACCACTC  
 501 GATGACCATG GTTCTGCTG TTGTCAGT TGGAGGATAC ACTCGATG  
 551 CCTGGTCCCTATTACCTC AACTTACTG TCCAGCTCTT CAGTACTAC  
 601 TACTACATGC GCTCCGTGC CGGTGTTGC ATCTGGTGG AGAGTACTT  
 651 GACCACTTC CAGATGCTC AGTGGTCTTGACCTTGAGGAGGAG  
 701 TCTGGCTCA CACCTACTTC GCTTCACTT ACTCCCTCTG GGATCCCAAC  
 751 GTGGCGAAGT GGCGGGTAC CGAGGGTGT GCCTCTTG GCTGGCAGT  
 801 CCTCTCCAGC TATCTCTTGC TCTTATCA CTTTACCGC ATTACCTACA  
 851 ATGCAAGGC CAAGGCAGGC TGGAGGCTG GAAGCAACTT TACCCCAAG  
 901 ACTGCAAGT CCGGGGAGTC GCGAGAGAG CCCTCAAGA GCAAGGACAT  
 951 CTAA

FIG. 6

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1 MAAILDKIN FGIDOPFGIK LDTYFADAYE LVTGSIOSF VFEQEVPLS  
 51 TQEAEVAMWII TYFVVFIFGR QIMKSDQAFK LKPFLTLHNF LLTIAAGSLL  
 101 LLFTENLVP1 LARGLFLAI CDDGANTQRL ELLYVLYLV KYMEADTVF  
 151 LVKKRPLEF LHYPFHHSMTM VLCFVQLGGY TSYSWMPITL NLTHVFRYY  
 201 YMRSAAIGR IWKAYLTLT QIVQFVLDLG FLYFCAATYF AFTYPMAPN  
 251 VGGCAGTEGA ALFGGGLSS YLLFNFYR ITYNAKAKAA KERGSNFTPK  
 301 TIKSGGSPKK PSKSKH1\*

FIG. 7

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1  
 GNS1 ~~~~~ M N S L V T Q Y A A P L F E R Y P Q L H D Y L P T I E R P F F I N I S S L W E I H F D D V  
 SUR4 M N T T T S T V I A A V A D Q F Q S L N S S S C F L K V H V P S I E N P F G I E L W P I F S K V  
 MAELO ~~~~~ M A A A I L D K V N F G I D Q P F G I K L D T Y F A Q A | 50

51  
 GNS1 V T R I V T N G E R F V P S E F Q F I A G E L P L S T L P P V L I A I T A Y V E I F G G R I F I L S K S  
 SUR4 F E Y F S G Y P A E Q F E F I H N K T F L A N G Y H A V I S I I T V Y T I I F G G Q A I L R A I L  
 MAELO Y E L V T G K S I D S F V F Q E G V T P L S T Q R E V A M W T I T Y F V V I F G G R Q I M K S Q | 100

101  
 GNS1 K P . F K L N G L F Q L H N L V L T S L S L T L L L M V E O L V P I I V O H G L Y F I A I G N I G  
 SUR4 S H A S P L K F K L L F E T H N L F T S I S L V L W L L M L E Q L V P M V Y H N G L F W S I G S K E  
 MAELO D A . F K L K P L F I L H N F L L T I A G S L L L F I E N L V P I L A R N G L F Y A I C D D G | 150

151  
 GNS1 A W T Q P L V T L Y Y M N Y I V K F I E C I D T F F L V L K H K K K L T F L H T Y H H G A T A L L G Y  
 SUR4 A F A P K L V T L Y Y L N Y L T K C V E L I D T V F L V L R R K K K L L F L H T Y H H G A T A L L G Y  
 MAELO A W T Q R L E L L Y Y L N Y L V K Y W E L A D T V F L V L K K K P L E F L H Y F H H S M T M V L G F | 200

201  
 GNS1 T I Q L M G T T S T S W V P I S L N L G V H V V M Y W Y Y F L I A A R G G I R V W W K E W V T R F Q I I Q  
 SUR4 T I Q L I G R T S V E W V V I L L N L G V H V V T M Y W Y Y F L I S S C G I R V W W K Q W V T R F Q I I Q  
 MAELO V Q L G G Y T S V S W V P I T L N L T V H V F M Y Y Y Y M R S A A G V R I W W K Q Y L T L Q I V Q | 250

251  
 GNS1 F V L D I G F I Y F A V Y Q K A V H L Y F P I L P H C G D C V G S T T A T I F A G C A I I S S Y L V  
 SUR4 F E I D L V F V Y F A T Y T F Y A H K Y L D G I L P N K G T C Y G T Q A A A A Y G Y L I L T S Y L L  
 MAELO F V L D L G F I Y F C A Y T Y F A F T Y P F W A P N V G K C A G T E G A A L F C C G L L S S Y L L | 300

FIG.8A

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301  
 GNS1 L F I I S F Y I N V Y Y K R K G T K T S R V V K R A H G G V A A K V N E Y V N V D L K N V P T P S P S P  
 SUR4 L F I I S F Y I Q S Y Y K K G G K K I V K K E S E V S G S V A S G S S T G V K T S N T K V S S R K A ~  
 MAELO L F I N F Y R I T Y N A K A K A K E R G S N F T P K T V K S G G S P K K P S K S K H I \* ~ ~ ~ | 350

351  
 GNS1 K P Q H R R K R  
 SUR4 ~ ~ ~ ~ ~ ~ ~  
 MAELO ~ ~ ~ ~ ~ ~ ~ | 400

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FIG.8B

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SCORES      Init1: 153 Initn: 199 opt: 495  
 57.4% identity in 549 bp overlap

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 MAELO      TCTGACCCAGAGGGCATGGCATCTTGGGATCACTACTTCGTCATCTTGGG  
 S78624      CATTAAGCACTTGGCCCTTGTGTATACCCATCACTGCTTATTATTG  
 5990      6000      150      160      170      180      190      200  
 MAELO      GTGCCAGATCATGAAAGGCCAG      6010      6020      210      220      230      240      250      260  
 S78624      GtGccAGGTTTGTAAAGTAACTGAAACCAATTAAATTAATGGCTTCCAAATT  
 6050      6060      6070      6080      6090      6100  
 MAELO      ACAACTCCCTCTGAGATGGTCC      6110      6120      270      280      290      300      310      320  
 S78624      ATATTGGTTAAC-TTACCTTATGAA-GGTTATGCTATGGTGAACATT  
 6130      6140      6150      6160  
 MAELO      GGCCCCATCTGCCAGAAACGGACTTCTACCTGCACTTCGGAGGCTCTGGAC  
 S78624      AGGCAAATTATGTTAGGACGGTTAACCTGCTTACTCTGTTACTGAAATTGGCTGAC  
 6170      6180      6190      6200      6210      6220  
 MAELO      390      400      410      420      430      440  
 S78624      CCAGGCCCTGAGCTCTACTACCTCAACTACTGGTCAAGTACTGGAGTTGGCA  
 6230      6240      6250      6260      6270      6280

500  
 MAELO      CACCGCTTTGGCTCTAGAGAGGGCTCTGGACTCTGGACTACTTCACCACTC  
 S78624      CACCTTTCTGGCTAAAGATAAAAATTGACATTGCA-TACT-ATCA-C  
 6290      6300      6310      6320      6330      6340

550  
 MAELO      GATGACCATGGTCTGCTTGT....CCAGCTGGAGATA-CACCTTCACTGCTGG  
 S78624      CAGGGCCTACGCTTATTAGTACACCCAAATTGATGGACCAACATTCTGG  
 6350      6360      6370      6380      6390      6400

610  
 MAELO      GTCCTTACCTCAACTGACTGTCAGCTCTCATGACTACTACATGGCTCC  
 S78624      GTCCTTACATGGCTGGATGTTGAGGATGGTACTTCAAAATTATCCAA  
 6460      6470      6480      6490      6500      6510

11/102  
 MAELO      TTCCCTTGGACCTGGATTCTACTCTGGCTACACCTACTTCGCTTCACTAC  
 S78624      GATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT  
 6520      6530      6540      6550      6560      6570

FIG. 9B

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| HOST(PLASMID)       | 334(pCGN7875)    | 334(pYES2)       | 334(pYX242)      | 334(pRAE-5)      | 334(pRAE-6)      | 334(pYX242)      | 334(pRAE-5)      |
|---------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| ADDED SUBSTRATE     | 25 $\mu$ M OA    | 25 $\mu$ M OA    | 25 $\mu$ M GLA   | 25 $\mu$ M GLA   | 25 $\mu$ M GLA   | NO SUBSTRATE     | NO SUBSTRATE     |
| FATTY ACID          | LIPID ( $\mu$ g) |
| C16:0               | 11.948           | 23.601           | 35.123           | 92.011           | 85.160           | 16.294           | 25.34            |
| C16:1               | 30.665           | 71.217           | 32.789           | 315.464          | 115.456          | 56.183           | 113.913          |
| C18:0               | 6.185            | 9.704            | 10.515           | 22.628           | 18.879           | 5.535            | 11.092           |
| C18:1n-9            | 35.340           | 57.429           | 33.989           | 154.386          | 106.881          | 28.388           | 51.538           |
| C18:3n-6            |                  |                  | 48.856           | 58.084           | 12.434           |                  |                  |
| C20:0               |                  |                  | 0.474            | 0.710            | 0.244            |                  |                  |
| C20:1n-9            | (0.375%)* 0.352  | (0.309%)* 0.527  |                  | 1.405            | 0.867            |                  | 0.516            |
| C20:3n-6            | ND               | ND               | (0.092%)* 0.226  | (0.324%)* 2.504  | (0.269%)* 1.006  | ND               | ND               |
| C22:0               |                  |                  |                  | 0.460            |                  |                  |                  |
| C22:1n-9            |                  |                  |                  | 0.321            | 0.315            |                  |                  |
| C24:0               |                  |                  |                  |                  | 1.825            |                  | 0.999            |
| TOTAL LIPID         | 93.760           | 170.490          | 245.090          | 771.690          | 374.420          | 112.99           | 256.52           |
| ND = NOT DETECTED   |                  |                  |                  |                  |                  |                  |                  |
| *% TOTAL FATTY ACID |                  |                  |                  |                  |                  |                  |                  |

FIG. 10A

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SUBSTITUTE SHEET (RULE 26)

| HOST(PLASMID)       | 334(pYX242)      | 334(pYX242)      | 334(pRAE-5)      | 334(pRAE-5)      | 334(pRAE-6)      |
|---------------------|------------------|------------------|------------------|------------------|------------------|
| ADDED SUBSTRATE     | 25 $\mu$ M GLA   |
| FATTY ACID          | LIPID ( $\mu$ g) |
| C16:0               | 60.683           | 61.487           | 100.998          | 96.193           | 66.761           |
| C16:1               | 79.838           | 79.586           | 359.754          | 220.440          | 87.359           |
| C18:0               | 9.784            | 10.106           | 15.317           | 15.165           | 16.744           |
| C18:1n-9            | 38.536           | 39.936           | 108.472          | 89.637           | 71.631           |
| C18:3n-6            | 17.974           | 17.833           | 82.866           | 56.596           | 17.766           |
| C20:0               |                  |                  | 0.510            | 0.570            |                  |
| C20:1n-9            |                  |                  |                  |                  |                  |
| C20:3n-6            | (0.136%)* 0.389  | (0.130%)* 0.374  | (0.336%)* 3.035  | (0.401%)* 2.689  | (0.353%)* 1.185  |
| C22:0               |                  |                  | 0.414            |                  |                  |
| C22:1n-9            |                  |                  |                  | 0.383            |                  |
| C24:0               |                  |                  | 1.513            | 1.626            |                  |
| TOTAL LIPID         | 285.560          | 288.045          | 902.560          | 671.113          | 335.496          |
| *% TOTAL FATTY ACID |                  |                  |                  |                  |                  |

FIG. 10B

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SUBSTITUTE SHEET (RULE 26)

| HOST(PLASMID)   | 334(pRAE-5/pCCR4) | 334(pYX242/pYES2) |  | HOST(PLASMID)   | 334(pRAE-5/pCCR4) | 334(pYX242/pYES2) |
|-----------------|-------------------|-------------------|--|-----------------|-------------------|-------------------|
| ADDED SUBSTRATE | 25 $\mu$ M GLA    | 25 $\mu$ M GLA    |  | ADDED SUBSTRATE | 25 $\mu$ M GLA    | 25 $\mu$ M GLA    |
| FATTY ACID      | LIPID ( $\mu$ g)  | LIPID ( $\mu$ g)  |  |                 | LIPID ( $\mu$ g)  | LIPID ( $\mu$ g)  |
| C16:0           | 41.050            | 37.169            |  | C16:0           | 96.986            | 32.221            |
| C16:1           | 99.393            | 100.552           |  | C16:1n-7        | 209.667           | 62.757            |
| C18:0           | 34.432            | 27.852            |  | C18:0           | 80.418            | 14.027            |
| C18:1           | 110.631           | 92.786            |  | C18:1n-9        | 207.104           | 28.701            |
| C18:3n-6        | 15.004            | 7.924             |  | C18:3n-6        | 25.264            | 10.543            |
| C20:0           | 0.643             | 0.574             |  | C20:0           | 2.038             |                   |
| C20:1           | 1.996             | 1.684             |  | C20:1n-9        | 3.591             |                   |
| C20:3n-6        | 0.542             | 0.607             |  | C20:3n-6        | 1.284             | 0.326             |
| C20:4n-6        | 0.579             |                   |  | C20:4n-6        | 1.394             |                   |
| C22:0           | 1.242             | 2.604             |  | C22:0           | 1.124             |                   |
| C24:0           | 4.754             | 4.563             |  | C24:0           | 3.952             |                   |
| TOTAL LIPID     | 334               | 300               |  | TOTAL LIPID     | 756               | 197               |

FIG. 11

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SUBSTITUTE SHEET (RULE 26)

| HOST(PLASMID)       | 334(pYX242)      | 334(pRAE-5)      | 334(pRELO-1)     | 334(pRELO-2)     |
|---------------------|------------------|------------------|------------------|------------------|
| ADDED SUBSTRATE     | 25 $\mu$ M GLA   |
|                     | 25°C/48HRS       | 25°C/48HRS       | 25°C/48HRS       | 25°C/48HRS       |
| FATTY ACID          | LIPID ( $\mu$ g) |
| C16:0               | 28.7             | 76.707           | 84.424           | 77.445           |
| C16:1               | 0.729            | 2.513            | 1.532            | 1.056            |
| C18:0               | 7.432            | 15.761           | 27.17            | 21.32            |
| C18:1n-9            | 28.9             | 77.323           | 109.419          | 82.844           |
| C18:3n-6            | 9.729            | 29.236           | 19.085           | 18.804           |
| C20:0               |                  | 0.643            | 0.522            | 0.537            |
| C20:1n-9            |                  | 0.77             | 0.426            | 0.299            |
| C20:3n-6            | (0.185%)* 0.374  | (0.279%)* 1.472  | (0.153%)* 0.748  | (0.200%)* 0.832  |
| C22:0               |                  | 0.451            |                  |                  |
| C22:1n-9            |                  |                  | 0.224            |                  |
| C24:0               |                  | 0.918            |                  |                  |
| TOTAL LIPID         | 202              | 527              | 490              | 416              |
| *% TOTAL FATTY ACID |                  |                  |                  |                  |

FIG. 12

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FIG. 13

| SCORES | Smith-Waterman score: | Init1:   | 178 | Initn: | 178 | Opt: | 318 | 33.0% | Identity in 188 aa overlap | 17/102 |
|--------|-----------------------|--|-----|--------|-----|------|-----|-------|----------------------------|--------|
| 268749 | MAELO                 | 50   | 60  | 70     | 80  | 90   | 100 |       |                            |        |
|        |                       | ELVTKSIDSFVQEGVTPISQREVAWMTTYFWVIGRQITMSQDAFLKPLFLHN           |     |        |     |      |     |       |                            |        |
|        |                       | 30   | 40  | 50     | 60  | 70   | 80  |       |                            |        |
| 268749 | MAELO                 | 110  | 120 | 130    | 140 | 150  | 160 |       |                            |        |
|        |                       | FFLVSFSYGSKTMPPF-MVQQIRLGLYGGCEAALSNPQAEYMLFLITLSKAVEFV        |     |        |     |      |     |       |                            |        |
|        |                       | 110  | 120 | 130    | 140 | 150  | 160 |       |                            |        |
| 268749 | MAELO                 | 90   | 100 | 110    | 120 | 130  | 140 |       |                            |        |
|        |                       | FLTTIAS-.GSLLLFIENYLPIARNGFLFAICD-GANTORLELLYLYKWMELA          |     |        |     |      |     |       |                            |        |
|        |                       | 170  | 180 | 190    | 200 | 210  | 220 |       |                            |        |
|        |                       | DTFFFLVLRKKPILFLHAYHHMATEVFFCSNYPTPSQSRVGVYINFLVHAFMPPYVFRS    |     |        |     |      |     |       |                            |        |
|        |                       | 150  | 160 | 170    | 180 | 190  | 200 |       |                            |        |
| 268749 | MAELO                 | 230  | 240 | 250    | 260 | 270  |     |       |                            |        |
|        |                       | MNIKUPAKISMANTVQLQLTQF...-MCFYIYGCTLMYSLATNQARYPSNTTATLQLCSTYL |     |        |     |      |     |       |                            |        |
|        |                       | 210  | 220 | 230    | 240 | 250  | 260 |       |                            |        |
| 268749 | MAELO                 | 280  | HLL |        |     |      |     |       |                            |        |
|        |                       | CGLLSYLLFLNFYRITYNAKAAKERRGSNFTPKTVSEGSPPKPKSKKHIX             |     |        |     |      |     |       |                            |        |
|        |                       | 270  | 280 | 290    | 300 | 310  |     |       |                            |        |

FIG. 14

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**SCORES** Init1: 30 Initn: 30 Opt: 40  
 Smith-Waterman score: 49; 22.1% identity in 86 aa over 1ap

|          |   |
|----------|---|
| AF003134 | MLYSITRRCYTFVTSLHRYQLYTECLEVNIVLNGOSINSRKD<br>10 20 30 40                       |
| MAELO    | MAAAILDKVNFGIDOPFGIKLDTYFAQ... YELVTKSIDSFVFCQEGV...PLSTQREV<br>10 20 30 40 50  |
| AF003134 | AEKITSFPFFM.....PQTFEQPHILTHLFFVFSVSLVTVFKKPKCEFPHSLA<br>50 60 70 80 90 100     |
| MAELO    | AMMTYFVWTFGGTQIMKSDAFLKLPLFTLHNFLLTIASGSULLFIEVNPVLARING<br>60 70 80 90 100 110 |

FIG. 15

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**Mouse SCORES** Init1: 161 Initn: 191 Opt: 325  
 Smith-Waterman score: 325; 28.8% identity in 285 aa over 1ap

|        |   |
|--------|---|
| U97107 | MTDSMNFSGKLKD...LNPYDFFTFOOLRPFLEYYWSSF.....LIVV<br>10 20 30 40 50 60   |
| MAELO  | MAAAILDKVNFGIDOPFGIKLDTYFAQ...ELVTKSIDSFVFCQEGV...PLSTQREVWVIT<br>50 60 70 80 90 100  |
| U97107 | YLLLIVGQTYKTRGFSLQRLWSFLAFLSGLTURMKINATMFTVGKQTV<br>TYFVTFGGTQIMKSDAFLKLPLFTLHNFLLTIAS...GSL...LLTENV...PILARNL<br>70 80 90 100 110             |
| U97107 | CFAYTODAVVRFWSFLLSKIV...ELGDTAFTILRKRPLIFVHRYHST...VLLFTS<br>FYATCDBGAWTQRLLELYLNLYKWMELDTFLVKKKPLEFLHVFHHSMTMVLCFVQ<br>110 120 130 140 150 170 |
| MAELO  | MAELO   |
| U97107 | 160 170 180 190 200 210   |
| MAELO  | FGTKKVKPSGEWF...MTNNFGHSMPTYYTMKAKUKHPIPLPMVTSIQLQMVIG...<br>LGYYTSV...SWPITLNLYAVMYYMMRSAGIR..IWKQYIITLQMFV...DLGF<br>180 190 200 210 220 230  |
| U97107 | 220 230 240 250 260   |
| MAELO  | TYFCAYTFFAFTYFPWAVNWKAGTEGAALFGCGLSSVLLLINFIRTY.NAKHAA<br>240 250 260 270 280 290   |
| U97107 | 270   |
| MAELO  | SKSQX<br>KERSNFTPKTVKSGGSPKSKSKHIX<br>300 310   |

Human SCORES

Init1: 147 Initn: 147 Opt: 211  
 Smith-Waterman score: 211; 28.7% identity in 150 aa over 1ap

|          |   |
|----------|---|
| MAELO    | NLVPILARNGLFYAICDDGAWTQRLLELYLNLYKWMELDTFLVKKKPLEFLHYFH<br>AC004050   |
| MAELO    | SLLVWDLTTLPICLPGDITILRKQLKLFHWH<br>10 20 30   |
| MAELO    | 170 180 190 200 210 220   |
| AC004050 | HSATMVLCFVQLGAYTSVSKWPTTNTLTHVFFYYMMRSAAGVTRWAK...QVLTTLQIV<br>40 50 60 70 80 90  |
| MAELO    | HITULLYSWNSYKDMVAGGGAFTMNYGHAVWNSYALRAGFGRVSRFAMFITSQIT<br>230 240 250 260 270 280  |
| AC004050 | QFVLDLGFIYFCAYTFAYTFWAPNWKAGTEGAALFGCGLSSVLLLINFIRTY<br>QMLNG...CVWNLVFC...WMDH...DCHSHFQNTIWMSSMlySLV...LCHFFFEAV<br>100 110 120 130 140 |

FIG. 16

| SCORES                | Init:   | 87    | Intr:    | 218               | Opt: | 232 | 20/102 |
|-----------------------|---|-------|----------|-------------------|------|-----|--------|
| Smith-Waterman score: | 272;  | 29.7% | identity | In 232 aa overlap |      |     |        |
| MAELO                 |   | 40    | 50       | 60                | 70   | 80  |        |
|                       | PRYSQRMVPPGQLHPVCLFCYLLTHAGTKTTEEPAVLPSIQLY         | 20    | 30       | 40                | 50   | 60  |        |
| MAELO                 |   | 100   | 110      | 120               | 130  | 140 |        |
|                       | LLLFTENLVTPLARGFLFYACDDGANTQRLELLY.-NYLKYWELAII     | 100   | 110      | 120               | 130  | 140 |        |
| MAELO                 |   | 80    | 90       | 100               | 110  | 120 |        |
|                       | .LYMFVLYTGIVMEGRYKNFQGTRSAEDSMKIRVWAWYFSKLIEFMM     | 80    | 90       | 100               | 110  | 120 |        |
| MAELO                 |   | 160   | 170      | 180               | 190  | 200 |        |
|                       | ..PLEFHYPFH-.SKTMVLCFVQLGGTYTSVSWPFTLNLTVHVFYYYYY-. | 160   | 170      | 180               | 190  | 200 |        |
| MAELO                 |   | 130   | 140      | 150               | 160  | 170 |        |
|                       | NHQITLVHVVYHATHMLNIAWVMMWNPFGHSYFGATLNSFTHLMLVSYGIG | 130   | 140      | 150               | 160  | 170 |        |

FIG. 17

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|     |            |            |             |               |            |
|-----|------------|------------|-------------|---------------|------------|
| 1   | MGTDQSKTTT | WEELAHNITK | DDLLAIRGR   | VVDTKFLSR     | HPGGDUTLL  |
| 51  | GAGRDTVPF  | ENYHAGAAD  | AIMKKYYGT   | LVSNELPIPF    | EPVFHKTK   |
| 101 | TRVEGYFTDR | NIDPKNRPET | WGRYALIFGS  | LJASYQAQLF    | VPFVIVERTL |
| 151 | QVYFAIMGF  | ACAQVGLNPL | HDASHFSVTH  | NPTWAKILGA    | THDFNGASY  |
| 201 | LVMMYQHMLG | HPHPTNIAGA | DPDVSTSEPD  | VRRKPNQKA     | FVNHINQHMF |
| 251 | VPEFLYGLAF | KIRQDINNL  | YFVKTDNDAIR | VNPISTMHTV    | MFWGGKAFFV |
| 301 | WYRLIVPLOY | LPIGLKVLFL | TVADMSSYY   | LALTQANH      | VEEVQHPLPD |
| 351 | ENGIIQDWA  | ANQVETTQY  | AHDSHLWTI   | TGSLYNQAH     | HLFPVNSQHJ |
| 401 | YPDIAITK   | TCSCEYKIVP | VKOTERDAEA  | SHI FHI RVI G | IRPKFEK*   |

FIG. 18

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SUBSTITUTE SHEET (B)(1) F 261

SUBSTITUTE SHEET (RULE 26)

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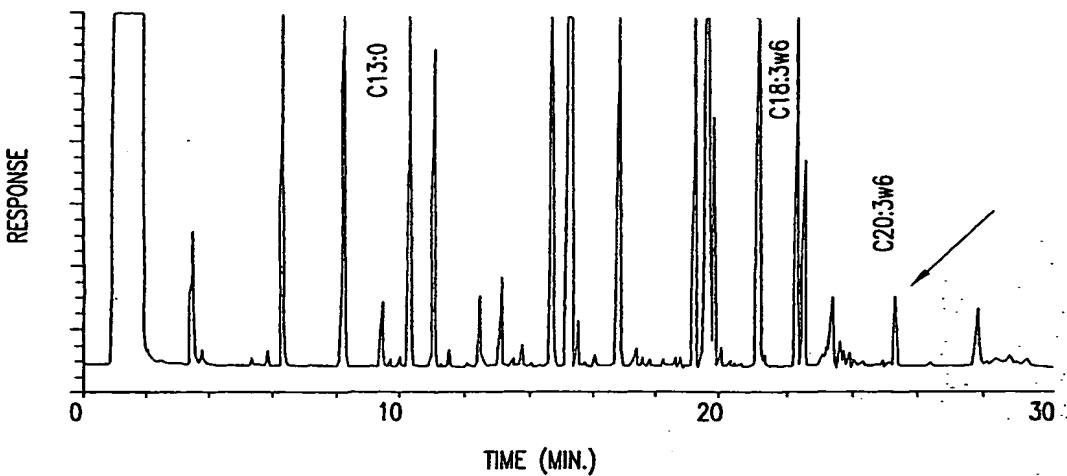


FIG. 19

SUBSTITUTE SHEET (RULE 26)

| HOST(PLASMID)                              | 334(MAD708-2)  | 334(MAD708-10) | 334(MAD708-18) | 334(MAD708-19) | 334(MAD708-30) | 334(pRAES)     |
|--|----------------|----------------|----------------|----------------|----------------|----------------|
| ADDED SUBSTRATE                            | 25 $\mu$ M GLA |
| FATTY ACID                                 | % TOTAL LIPID  |                |                |                |                |                |
| C16:0                                      | 14.1           | 14.68          | 14.38          | 15.45          | 14.13          | 13.59          |
| C16:1                                      | 42.84          | 43.42          | 42.57          | 38.03          | 43.58          | 43.98          |
| C18:0                                      | 3.19           | 3.28           | 3.63           | 4.08           | 3.37           | 2.04           |
| C18:1n-9                                   | 17.66          | 19.39          | 19.6           | 20.8           | 20.06          | 10.88          |
| C18:3n-6                                   | 6.65           | 5.58           | 10.24          | 9.46           | 3.56           | 11.14          |
| C20:0                                      | 0.26           | 0.3            | 0.32           | 0.4            | 0.46           | 0.57           |
| C20:3n-6                                   | (47.5%) 6.03   | (41.2%) 3.92   | (8.0%) 0.91    | (21.5%) 2.59   | (49%) 3.43     | (3.4%) 0.24    |
| TOTAL LIPID ( $\mu$ g)                     | 238.47         | 307.86         | 188.51         | 167.31         | 207.47         | 466.65         |
| (% CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT) |                |                |                |                |                |                |

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FIG. 20

SUBSTITUTE SHEET (RULE 26)

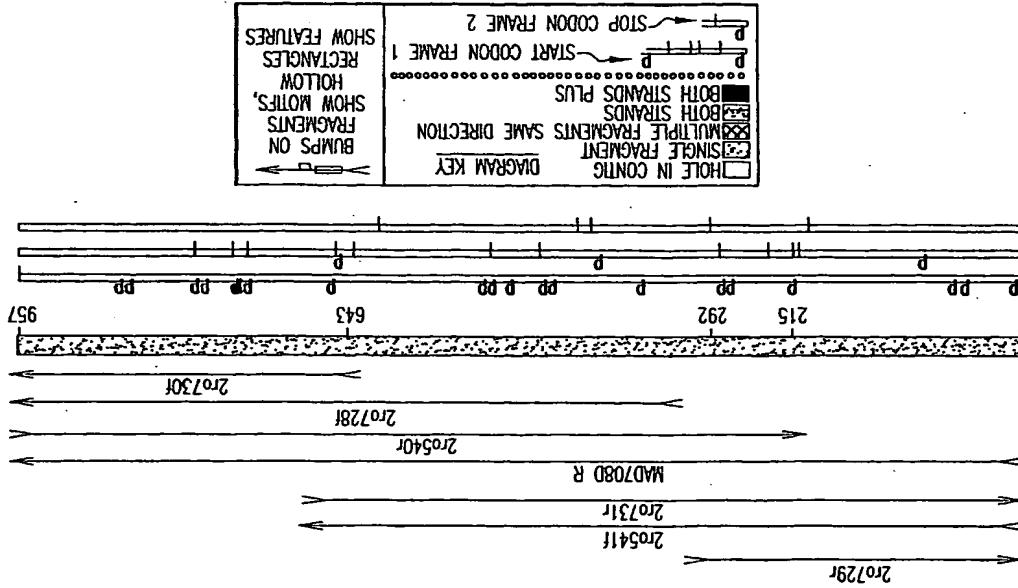
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1 MEAVVNLQE VNIKHAQDPIQ GYPLMGSPLL MTSILLTYYV FVLSIGPRIM  
 51 ANRKPFOLRG FIVVNFSLV ALSIYTVEF LMSGWLSYT MRCPPDVSN  
 101 SPEALRMVY AHIIFLSKF1 ELMDTVIFL RKKDQVTF1 HVFHHSVLPW  
 151 SWANGUKIAP GEMGSFHANI NSSVHIMYL YIGLSAFGPRV ADPQYMKKKH  
 201 MTAIQLIQFV LVSLSHSQYF FMSSCNQYP VITHLIMNG TIFFNLSNF  
 251 WHHSYTKGKR LPRALQNGA PGIAKVKAN\*

FIG. 22

FIG. 21



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|     |  |
|-----|--|
| 1   | MESIAFLPS KNPQDLFMDL ATAIGRAAP YDPLDEALV ADAEKYIPTI    |
| 51  | WHTRGFLVA VESPLARELP LMNPFHILLI VLAYLVTFV GHQIMKNER    |
| 101 | FEVKTSLL E NFLCVSISAY NGGTLYEY QANYGLFENA ADHTFKGLPM   |
| 151 | ANMIMLFYS KIMEFVDMI NVLKKNNRQJ SPLHVYHSS IFTIWLVTF     |
| 201 | VAPNGEAYFS AALNSFIHVJ NYGYYFLSAL GFKQVSFIKF YITRSQMTQF |
| 251 | CMMSVQSSWD MYAMKVLRP GYPFFITALL WFYMMTMLGL FYNFYRKNAK  |
| 301 | LAKOAKADAA KEKARKLO*                                   |

FIG. 23

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FIG. 24

FIG. 25A

| HOST(PASMD)     | 334(pRB2) | 334(pRB2) | 334(pRB2) | 334(pRB2) | % TOTAL UpD10 | FATTY ACID  |
|-----------------|-----------|-----------|-----------|-----------|---------------|-------------|
| ADD'D SUBSTRATE | 25M SA    | 25M OA    | 25M LA    | 25M DOL   | 25M MA        | 25M ARBENIC |
| C16:0           | 15.07     | 14.52     | 15.74     | 15.69     | 16.06         | 15.15       |
| C16:1           | 33.7      | 32.37     | 32.23     | 25.65     | 33.65         | 33.39       |
| C18:0           | 33.7      | 32.37     | 32.23     | 25.65     | 33.65         | 33.39       |
| C18:1n-9        | *9.78     | 5.83      | 5.61      | 8.33      | 4.52          | 5.35        |
| C18:2n-6        | 31.2      | *37.25    | 26.05     | 20.15     | 24.54         | 28.54       |
| C18:3n-6        |           |           |           | *10.4     |               |             |
| C20:2n-6        |           |           |           | 0.29      |               |             |
| C20:3n-6        |           |           |           | *16.5     |               |             |
| C20:4n-6        |           |           |           | 0.27      | *11.7         | *7.46       |
| C22:4n-6        |           |           |           |           |               | 163         |
| TOTAL UpD (mg)  | 132       | 130       | 171       | 55        | 225           |             |

FIG. 25B

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| HOST(PLASMID)          | 334(pRFB2+PRPE31) | 334(pYES2+PRX242) |
|------------------------|-------------------|-------------------|
| ADDED SUBSTRATE        | 25 $\mu$ M GJA    | 25 $\mu$ M GJA    |
| FATTY ACID             | % TOTAL LIPID     |                   |
| C16:0                  | 15.54             | 18.26             |
| C16:1                  | 30.16             | 33.51             |
| C18:0                  | 8.76              | 5.58              |
| C18:1n-9               | 27                | 27.37             |
| C18:3n-6               | *5.6              | *5.6              |
| C20:0                  | 0.4               | 0.32              |
| C20:3n-6               | (57.4%) 3.55      | (2.9%) 0.17       |
| C20:4n-6               | (27.6%) 1.32      | ND                |
| TOTAL LIPID ( $\mu$ g) | 254               | 258               |

\*INDICATES SUBSTRATE ADDED  
(% CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT)

FIG.26A

| HOST(PLASMID)          | 334(pRFB2+PRPE31) | 334(pYES2+PRX242) |
|------------------------|-------------------|-------------------|
| ADDED SUBSTRATE        | 25 $\mu$ M STA    | 25 $\mu$ M STA    |
| FATTY ACID             | % TOTAL LIPID     |                   |
| C16:0                  | 18                | 16.4              |
| C16:1                  | 28.37             | 34.78             |
| C18:0                  | 7.42              | 5.71              |
| C18:1n-9               | 26.44             | 30.15             |
| C18:4n-3               | *2.93             | *4.57             |
| C20:0                  | 0.25              | 0.17              |
| C20:4n-3               | 4.13              | 0.32              |
| C20:5n-3               | (39%) 1.87        | (2.1%) 1.0        |
| TOTAL LIPID ( $\mu$ g) | 257               | 304               |

\*INDICATES SUBSTRATE ADDED  
(% CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT)

FIG.26B

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SCORES Int'l: 114 Smith-Waterman score: 308; Opt: 278  
Smith-Waterman score: 308; 30.9% identity in 259 aa over 1 ap

GLELO VAOAEKTYPTIVHTRGFVAVESPLAREPLPUNPFLVAVLVLTVFVQHQMKNFE  
MAELO GIKLDTTYFAQAYELVTGKSDSEFQEQTPLSTOREAMWTTYFWVIFGGRQLMSQD  
GLELO REEWKTFSLHNFLCLVSAYMCGGLIVE-AYQANYGLFENVAAHTEFKGLPMAKWMILF  
MAELO AFKLKPFLFHNLFLTIASSLLFIENLPIYLARGLFLVACDGAWTORLELLYYLN  
GLELO YFSKINEFYDTHMVLKKNRQTSFLHVYHASSIFTIMLVTFVAPNGEAYFSAALNSFT  
MAELO YLWKYMELADTVFLVLUK-K-KEPLFLHYNHIS-ATPWULCFVQLGGYTSVSWPITLTV  
GLELO HVWMYGYFLSALGFQKVSITKIVTRSONTQF-S...-CMNSVQS-S...-SMDVYAM  
MAELO HVFRYYMMRSAGVRI-WMKQNLTTLQIVQFLDGFYTFAYTYFARTYFPMWAPNVG  
GLELO KVLRPGYPFFITLWYMMTFLGFLYFNRKNAKQAKADAKEKARKLQ  
MAELO KCAGTEGAALFGGLSSYLL-LFINFYR-ITYNAKAKAKERSNFTPTKVK  
MAELO GGSPKKPSKSKHIX

FIG.27

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|       |     |   |                                       |    |
|-------|-----|---|---------------------------------------|----|
| GLELO | 1   | - M E S I A P F L P S K M P Q D L F M D L A T A I G V R A A P Y V D P L E A A L V A Q   | 42                                    |    |
| MAELO | 1   | - - - - -   | MAA A I L D K V N F G I D Q P F G I K | 20 |
| GNS1  | 1   | - - - - - M N S L V T Q Y A A P L F E R Y P Q L H D Y L P T L E R P F F N I S L W       | 36                                    |    |
| SUR4  | 1   | M N T T S T V I A A V A D Q F Q S L N S S S C F L K V H V P S I E N P - F G I E L       | 42                                    |    |
| GLELO | 43  | A E K Y I P T I V H H T R G F L V A V E S P L A R E L P L M N P F H V U L L I V L A Y L | 85                                    |    |
| MAELO | 21  | L D T Y F A Q A Y E L V T G K S I D S F V F Q E G V T P L S T Q R E V A M W T I T Y F   | 63                                    |    |
| GNS1  | 37  | E H F D D V V T R V T N G R F V P S E F Q F I A G E L P L S T L P P V L Y A I T A Y Y   | 79                                    |    |
| SUR4  | 43  | W P I F S K V F E Y F S G - Y P A E Q F E F I H N K I F L A N G Y H A V S I I I V Y Y   | 84                                    |    |
| GLELO | 86  | V T V F V G M Q I M K N F E R F E V K T F S L L H N F C L V S I S A Y M C G G I L Y E   | 128                                   |    |
| MAELO | 64  | V V I F G G R Q I M K S Q D A - - F K L K P L F I L H N F L L T I A S G S L L L L F I   | 104                                   |    |
| GNS1  | 80  | V I I F G G R F L L S - - K S K P F K L N G L F Q L H N L V L T S L S T L L L L N V     | 120                                   |    |
| SUR4  | 85  | I I I F G G Q A I L R A L N A S P L K F K L L F E I H N L F L T S I S L V L W L L M L   | 127                                   |    |
| GLELO | 129 | - - A Y Q - - A N Y G L F E N A A D H T F K G L P M A K M I W L F Y F S K I M E F V D   | 167                                   |    |
| MAELO | 105 | E N L V P I L A R N G L F Y A I C D D G A W T Q R L E L L Y Y L N Y L V K Y W E L A D   | 147                                   |    |
| GNS1  | 121 | E Q L V P I I V Q H G L Y F A I C N I G A W T Q P L V T L Y Y M N Y I V K F I E F I D   | 163                                   |    |
| SUR4  | 128 | E Q L V P M V Y H N G L F W S I C S K E A F A P K L V T L Y Y L N Y L T K F V E L I D   | 170                                   |    |
| GLELO | 168 | T M I M V L K K N N R Q I S F L H V Y H H S S I F T I W W L W T F V A P N G E A Y F S   | 210                                   |    |
| MAELO | 148 | T V F L V L K K - - K P L E F L H Y F H H S M T M V L C F - V Q L G G Y T S V S W V P   | 187                                   |    |
| GNS1  | 164 | T F F L V L K H - - K K L T F L H T Y H H G A T A L L C Y - T Q L M G T T S I S W V P   | 203                                   |    |
| SUR4  | 171 | T V F L V L R R - - K K L L F L H T Y H H G A T A L L C Y - T Q L I G R T S V E W V V   | 210                                   |    |

FIG.28A

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|       |     |   |     |
|-------|-----|---|-----|
| GLELO | 211 | A A L N S F I H V I M Y G Y Y F L S A L G F K Q V S F I K F Y I T R S Q M T Q F C M M | 253 |
| MAELO | 188 | I T L N L T V H V F M Y Y Y M R S A A G V R - - I W W K Q Y L T T L Q I V Q F V L D   | 228 |
| GNS1  | 204 | I S L N L G V H V V M Y W Y Y F L A A R G I R - - V W W K E W V T R F Q I I Q F V L D | 244 |
| SUR4  | 211 | I L L N L G V H V I M Y W Y Y F L S S C G I R - - V W W K Q W V T R F Q I I Q F L I D | 251 |
| GLELO | 254 | S V Q S S W D M Y A M K V L G R P G Y P F F I T A L L W F Y M W T M L G L F Y N F Y R | 296 |
| MAELO | 229 | L G F I Y F C A Y T Y F A F T Y F P W - A P N V G K C A G T E G A A L F G G C L L S S | 270 |
| GNS1  | 245 | I G F I Y F A V Y Q K A V H L Y F P - I L P H C G D C V G S T T A T F A G C A I I S S | 286 |
| SUR4  | 252 | L V F V Y F A T Y T F Y A H K Y L D G I L P N K G T C Y G T Q A A A A Y G Y L I L T S | 294 |
| GLELO | 297 | K N A K L A K Q A K A D A A K E K A R K L Q   | 318 |
| MAELO | 271 | Y L L L F I N F Y R I T Y N A K A K A A K E R G S N F T P K T V K S G G S P K K P S K | 313 |
| GNS1  | 287 | Y L V L F I S F Y I N V Y K R K G T K T S R V V K R A H G G V A A K V N E Y V N V D L | 329 |
| SUR4  | 295 | Y L L L F I S F Y I Q S Y K K G G K K T V K K E S E V S G S V A S G S S T G V K T S N | 337 |
| MAELO | 314 | S K H I   | 317 |
| GNS1  | 330 | K N V P T P S P S P K P Q H R R K R   | 347 |
| SUR4  | 338 | T K V S S R K A   | 345 |

FIG.28B

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SCORES Init1: 83 Initn: 186 Opt: 271  
 Smith-Waterman score: 297; 28.5% identity in 242 aa overlap

SCORES Init1: 88 Initn: 208 Opt: 272  
Smith-Waterman score: 279; 28.2% identity in 266 aa overlap

|       |   |                                  |
|-------|---|----------------------------------|
| MAELO | 30<br>QAYELVTKSIDSFLFQEGVTPLSQREAVWMTYFVIFGGQIIMKSQDAFKLKLKF1   | 40<br>50<br>60<br>70<br>80       |
| HS2   | VNLQEVWIKHADPRQGYPGLMSPLMTSILTVYFVLISLGPR-IMANRKPFQLRGFMI       | 10<br>20<br>30<br>40<br>50<br>60 |
| MAELO | 90<br>LHNFLTTIASGLLULFIEN-LVPIALAR-NGLFVAICDGAICWQRLELYLNLVYKWF | 100<br>110<br>120<br>130<br>140  |
| HS2   | 70<br>VNNFSEVSLVTEELWMSGULSTTTCRCPVDSIYSSPEALRNVRVWHLF5-CKF1    | 80<br>90<br>100<br>110<br>120    |

|       |   |     |     |     |     |     |     |     |     |     |     |     |     |
|-------|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| MAELO | ELADTYFELVKKK...PLEFHYPFHSMT...-WVLCFWFLQGGTTSVSVWPTILNLTVHVF | 130 | 140 | 150 | 160 | 170 | 180 | 190 | 200 | 210 | 220 | 230 | 240 |
| HS2   | ELMDTVFILRKDKQVTFLHVFHSSVLPWSMAGVKAPGMSGFHAM...-INSSVWV       | 130 | 140 | 150 | 160 | 170 | 180 | 190 | 200 | 210 | 220 | 230 | 240 |
| MAELO | MYYYMMRSAGY...-RTIWAQYLTTLQIQVFLV...-DLGTFYF...-GATYTFAYTFPW  | 130 | 140 | 150 | 160 | 170 | 180 | 190 | 200 | 210 | 220 | 230 | 240 |
| HS2   | MYLYYGLSAGFGRVAPYIWLWAKKHMATAQLOFVLUVSHISQYPMSSCQYQYVTHILW    | 130 | 140 | 150 | 160 | 170 | 180 | 190 | 200 | 210 | 220 | 230 | 240 |

|     |  |
|-----|--|
| ELO | ALFGCGLSSYLLFINFRTYNNAKAAKERSNFTPTKVGSSPKPKSKHII       |
| 1   | LYFIGMISLIALFTNFYIOTYNNKAGSRKOHLDHQNGMSAUVNGHTNSPLENNV |

KPRKLRKDX 300

MAELO  
310  
PKKPSKSKHI

FIG. 25

X 280

FIG. 30

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SSCORES Init1: 88 Initn: 207 Opt: 223 Smith-Waterman score: 236; 30.4% identity in 191 aa overlap

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Smith-Waterman score: 168;  $\text{E-Value: } 1.12 \times 10^{-10}$   $\text{Identity: } 30.4\%$   $\text{Overlap: } 115$  aa overlap

FIG. 31

FIG. 32

| SCORES   | Init1: 51   | Initn: 115 | Opt: 168 | Smith-Waterman score: 168; | 30.4% identity in 115 aa overlap | 37/102 |
|----------|---|------------|----------|----------------------------|----------------------------------|--------|
| MAELO    | 30  | 40         | 50       | 60                         | 70                               | 8      |
|          | YELVGTGSDTSFVFGEGVPLSTOREVAMTTYFWVFIGGRQIMKSDQAF      |            |          |                            |                                  |        |
| A1225632 | NAFDWIFGPDRSIVGAFELDOSYLPPTFLTITIYLISWLGNKYMKIRPAl    | 20         | 30       | 40                         | 50                               | 60     |
| MAELO    | 90  | 100        | 110      | 120                        | 130                              |        |
|          | NFLLTIASSLLLFENIVPLAARGFLFYACDD...GANTORLELYYY        |            |          |                            |                                  |        |
| A1225632 | NLATISSLASVMLVELI.....LSSMEEGGYNLQCNQDLSAGEGDIYRVAKVW | 80         | 90       | 100                        | 110                              |        |
| MAELO    | 150   | 160        | 170      | 180                        | 190                              |        |
|          | LADTIVELVKKK...PFLFLHYFHAKNTMTCVQEGGTTSYSKAPITNLIT    |            |          |                            |                                  |        |
| A1225632 | FLDTITIFFVLRKKAQNQTFHLHYVHMASMFI                      | 130        | 140      | 150                        |                                  |        |

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SCORES Frame: (3) Init1: 332 Initn: 332 Opt: 384  
40.3% Identity in 144 aa overlap

GLE0 LIVLAYLVTIVFGQINQKINFEREKVTFSSLHNFCLVLSAYNCGGILYEAVQANIGL·F  
A1815960 |:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:  
LYNLGITLISAYMLAEIILISTMEGGYNLOC  
10 20 30

140 150 160 170 180 190  
GLE0 ENAADHTFKGIPHAKMILWLFESKIMEFVDTMMWIKKNNRQISFLHVYTHSSIFTIWL  
A1815960 ::|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:  
QDLTSAEADIRVAKVLWVYFSKSVNEFLDTIFFLRKTSQITLHVYHASMFNIMAC  
40 50 60 70 80 90

200 210 220 230 240 250  
GLE0 VTFVAPNGEAVFSAALNSFHVHMYGYFESAL·GFKQNSFIKFYITRSOMTQFCMMSVQ  
A1815960 |:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:  
VLNWIPCGQSFEGPTLNSFTHILMYSYGLSVFSPSNHKLWIKYLTQQLVQF  
100 110 120 130 140

260 270 280 290 300 310.  
GLE0 SSADMYANKVLRGPGYFFITALLWFMATMGLFYNFVKVAKLAKQAKADAKEARK

**FIG. 33**

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SCORES Init1: 316 Initn: 384 Opt: 477  
Smith-Waterman score: 477: 34.2% identity in 240 aa overlap

GLE0 AQAEKTYPTIVHTRGFIVAVESPLAREPLMPMFHLLIVLAXLVTVFGQINQKINFER  
HS1 |:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:  
MEHDASLSTYFKALLGPRDTRKGFELDNYPTFCISVYLLWGPKYKRNKP  
10 20 30 40 50

110 120 130 140 150  
GLE0 FEYKTFSLIHNFLCLVTSAYMCGGILYEAOQNYGLF·ENAADHTFKGLPMKMMWLFYF  
HS1 |:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:  
FSCRGILVWVNLGLTLLSLYMFCELVTGWEKGKYNFFCGTRTAGESDNKIRVLMWYF  
60 70 80 90 100 110

160 170 180 190 200 210 219  
GLE0 SKTMEFDTMIVNLKKNNRQISFLHVYHASSFTTIVLWTFVAPNGEAVFSAALNSFHV  
HS1 |:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:  
SKLIEPMDTFFTRKNNHQITLVHVYHASNLTWFMWMMVPGHSYFGTLSISFHV  
120 130 140 150 160 170

220 230 240 250 260 270  
GLE0 INGYYFLSAL·GFKQNSFIKFYITRSOMTQFCMMSVQSSADMYANKVLRGPGYFFITA  
HS1 |:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:  
LMSYGLSSVPSMRPLWIKYLTQQLVQFVLTIQTS····CGVWPCFTFP·GWLY  
180 190 200 210 220 230

280 290 300 310  
GLE0 LLWFMATMGLFYNFYRK·-NAKLAKQAKADAKEARKLQ  
HS1 |:|:|:|:|:|:|:|:|:|:  
FQGYNISLIALFTNFYIQTYYKGSRKOHQKHSMAAVNGHTNSFSPLENNVP  
240 250 260 270 280 290

**FIG. 34**

| SCORES   | Init1: 80   | Initn: 114 | Opt: 178 | Smith-Waterman score: 178; | 28.8% identity in 146 aa overlap | 40/102 |
|----------|---|------------|----------|----------------------------|----------------------------------|--------|
| GLELO    | FENAAHTFKGLPMKAMWILFYSKIMEFVDTMINLKKQRQISFLHVYHSSLFTIWW       | 140        | 150      | 160                        | 170                              | 180    |
| AC004050 | DTTFLILRK...QKLFLHMYHHTVLYSW                                  |            |          |                            |                                  |        |
| GLELO    | LVTFAVPNGEAYFSAALAINSETHIIMGYYFLSALGFKQVSFKFVITRSQQTQFCMMSVQ  | 200        | 210      | 220                        | 230                              | 240    |
| AC004050 | YSTKDNVAGGGWF...MTMNGVHAWMSSYALRAAGFYSRKAMFTISQITQMLMGEV      | 30         | 40       | 50                         | 60                               | 70     |
| GLELO    | SSMDWYAKVYLRGPYFFITALLW...FTWMMTMLGLFVWTRKNA...AKLADQAKADAAKE | 260        | 270      | 280                        | 290                              | 300    |
| AC004050 | NYLVFCMHQ...HQDQCHF...QNTFSSMLSYLVLFCHFFEAIGKMRKTTKAEX        | 90         | 100      | 110                        | 120                              | 130    |
| GLELO    | KARKLQ  |            |          |                            |                                  |        |

FIG. 35

| SCORES | Init1: | 288 | Initn: | 288 | Opt: | 399 | Smith-Waterman score:   | 399; | 34.6% identity in 211 aa overlap | 41/102 |     |     |     |
|--------|--------|-----|--------|-----|------|-----|---|------|----------------------------------|--------|-----|-----|-----|
| GLEL0  | 80     | 90  | 100    | 110 | 120  | 130 | LLTVLAYLTVFNGQIMKNNPERFEVKTFSLLNHNFCLNISIAYMCGGILYAYAQANGLF<br>:::   :::   :::   :::   :::   :::   :::   :::   :::   :::   :::   :<br>IVNFSVLVLSLYIWFELNSGALSTYTWR                                    | 10   | 20                               | 30     |     |     |     |
| MN2    |        |     |        |     |      |     |   |      |                                  |        |     |     |     |
| GLEL0  | 140    | 150 | 160    | 170 | 180  | 190 | ENAAAD -HTFKGLPAAKATWLFYFSKINMEFDTHMVKKNNRQTSFLHIVYHASSIFTIW<br>:::   :::   :::   :::   :::   :::   :::   :::   :::   :::   :::   :<br>COPIDFSNSPEALRWRVIAWLFMLSKVTELMDTVTFLURKGQAVTFLHIVFHSVLPWSW    | 40   | 50                               | 60     | 70  | 80  | 90  |
| MN2    |        |     |        |     |      |     |   |      |                                  |        |     |     |     |
| GLEL0  | 200    | 210 | 220    | 230 | 240  | 250 | WLVTIVYAPNGEAYFSAALNSFTHITMYGGYELSAIGFQVSEFI ..KFTVITRSQNTQFCMN<br>:::   :::   :::   :::   :::   :::   :::   :::   :::   :::   :::   :<br>WAGKIKIAPGGMGSFHAMINSSHVWMMLYGGLSAGPVAQPYLWAKHMMTAIQLIQFVLV | 100  | 110                              | 120    | 130 | 140 | 150 |
| MN2    |        |     |        |     |      |     |   |      |                                  |        |     |     |     |
| GLEL0  | 260    | 270 | 280    | 290 | 300  | 309 | SVQSSDIDYAMKVLGRGPYIPFTTALLFVYWTMGLFYNF.....YRKNAKLQAKADA<br>:::   :::   :::   :::   :::   :::   :::   :::   :::   :::   :<br>SLHIS QYFMPSCNQY .YPVIIH-LIMMYGTIFFILESNEFWHYSYTRGKRLPRAVQONG           | 160  | 170                              | 180    | 190 | 200 |     |
| MN2    |        |     |        |     |      |     |   |      |                                  |        |     |     |     |
| GLEL0  | 310    |     |        |     |      |     | AKEKARKLQ   |      |                                  |        |     |     |     |
| MN2    |        |     |        |     |      |     | APATTKVKAN  |      |                                  |        |     |     |     |

FIG. 36

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SCORES Init1: 160 Initn: 227 Opt: 269  
 Smith-Waterman score: 269; 35.3% identity in 119 aa overlap

50 60 70 80 90 100  
 PTIVHHTRGFLVAVESPLAREPLPMLPFLVLTIVLAVLTVTIVGQIWKANPEREVKTF  
 :::::|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:  
 AI225632 NEVNAFLDINMFGFGRDSRVRGWFELDSYLPFTILTSLTIVLGIKYYRNPAISLRGTL  
 10 20 30 40 50 60

110 120 130 140 150 160  
 LLHNFCCVTSAMCGGILYEAYQANYGLFENAAADHTFG-LPMAG-MWLFYFSKTMIEF  
 ::|:|:|:|:|:  
 AI225632 TLYNLAITLTLAIVLVELISSLSEGGYNLQCONLSDAGEGDIVRAVKLVW-YFFSKLVEF  
 70 80 90 100 110 120

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SCORES Init1: 160 Initn: 227 Opt: 269  
 Smith-Waterman score: 239; 23.7% identity in 279 aa overlap

50 60 70 80 90 100  
 PTIVHHTRGFLVAVESPLAREPLPMLPFLVLTIVLAVLTVTIVGQIWKANPEREVKTF  
 :::::|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:  
 AI225632 NEVNAFLDINMFGFGRDSRVRGWFELDSYLPFTILTSLTIVLGIKYYRNPAISLRGTL  
 10 20 30 40 50 60

110 120 130 140 150 160  
 LLHNFCCVTSAMCGGILYEAYQANYGLFENAAADHTFG-LPMAG-MWLFYFSKTMIEF  
 ::|:|:|:|:|:  
 AI225632 TLYNLAITLTLAIVLVELISSLSEGGYNLQCONLSDAGEGDIVRAVKLVW-YFFSKLVEF  
 70 80 90 100 110 120

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SCORES Init1: 64 Initn: 129 Opt: 233  
 Smith-Waterman score: 239; 23.7% identity in 279 aa overlap

50 60 70 80 90 100  
 PTIVHHTRGFLVAVESPLAREPLPMLPFLVLTIVLAVLTVTIVGQIWKANPEREVKTF  
 :::::|:|:|:|:|:|:|:|:|:|:|:|:|:|:  
 AI225632 NEVNAFLDINMFGFGRDSRVRGWFELDSYLPFTILTSLTIVLGIKYYRNPAISLRGTL  
 10 20 30 40 50 60

110 120 130 140 150 160  
 LLHNFCCVTSAMCGGILYEAYQANYGLFENAAADHTFG-LPMAG-MWLFYFSKTMIEF  
 ::|:|:|:|:|:  
 AI225632 TLYNLAITLTLAIVLVELISSLSEGGYNLQCONLSDAGEGDIVRAVKLVW-YFFSKLVEF  
 70 80 90 100 110 120

FIG. 37

GLELO 20 30 40 50 60  
 FMOLATAIGVRAAPYDPLEAALVAQAEKYIPTIVHHTRGFLVAVESPLAREL...PL  
 ::|:|:|:|:  
 U97107 MOTSMNSRGLKMDLMQPYDFETFQQLRPF  
 10 20 30

80 90 100 110 120 129  
 MNPFHV-LIIVLALYLVTFVGHQIWKANPEREVKTFSLHHNFCVLISAYMGGGLYEA  
 ::|:|:|:|:  
 U97107 LEEWWSFLLIVWVLLIWGATYMRTRKSFLQRPLIWSFFLAIFS-ILGTRMK  
 40 50 60 70 80

130 140 150 160 170 180  
 YQAN...YGLFENAAADHTFG-LPMAG-MWLFYFSKTMIEF-LFYFSKIMEFVDTIMWLLKANRQISFL  
 ::|:|:|:|:  
 U97107 FMATVMTFVGKQIVCFAITDIAVIRF-WSFVFLSKVNLGDTAFTILRK--RPLIFV  
 90 100 110 120 130 140

190 200 210 220 230 240  
 HYYHHSST-FTIWWLVTFEVAPNGEAYFSAAIINSFHVIMGYFY-SALGFKVSEFKFY  
 ::|:|:|:|:  
 U97107 HWYHHSSTLFLTSEGIKVKY-PSSGFMFT-MNFGIHSWVNTYTTKAACKLHPNLLPWW  
 150 160 170 180 190 200

250 260 270 280 290  
 ITRSQMTQFCMSVQSSWWDYVANKVLG-RPGYFFITALLWYMTMGLFVN-FYRK  
 ::|:|:|:|:  
 U97107 ITSLQIQLWVLTGTFGLNTYARQEKGCHTTTEFFMSFLYGTYFLFLFAHFFHRYLRP  
 210 220 230 240 250 260

300 310  
 NAKLAQAKADAKEKARIKLQ  
 ::|:|:|:  
 U97107 KGKVASKSQ  
 270

FIG. 38

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SCORES Init: 100 Init: 205 Opt: 271 Smith-Waterman score: 271; 30.7% identity in 218 aa overlap

GLE0 TRGFVAVESPLAREPLPLMNPFLVFLVTLVFLVTFGMQJMKNFRERFKTFSLLNFC 60 70 80 90 100 110  
U68749 ATHGPKNFPDAEGRKFFADHFDITDQASILYMWVNGTKMFRMRIRQFQLTTPLNTNFI (F56H11.4) 30 40 50 60 70 80.

GLE0 LVTSAYMCGGILYEATQ.-ANYGL...FENAAQHTEFKGLPMAKMINLFLYFSKIMFVDT 120 130 140 150 160  
U68749 LAAFSIAGAAMRMPFFECTIANGIVASCYCKVFDT-KG-ENGMWMLFMSKLFEVDT (F56H11.4) 90 100 110 120 130 140.

GLE0 MIMVLUKNNRQISFLHYHHSISITTMWLVTFAPNGEAVFSAALNSFIRINVGYFFLS 170 180 190 200 210 220  
U68749 IFLVLRK -RPLMFLHNYHHTLTYIAYNSHPLP -GFNRYGYTLNFVHAFNYSYFLR (F56H11.4) 150 160 170 180 190.

GLE0 ALGKQUSFKTFYITRSQMTFCMNSQSSHAKVKGPF -GYFFITALLWYWTM 230 240 250 260 270 280  
U68749 SMK1RVPFQIAQTSLOQNOF11SCAVLAHLGUMHFTNACDFEPVFKLAVFDITV (F56H11.4) 200 210 220 230 240 250.

GLE0 LGLFNFYRKMAKLQAKADAAKEKARKLQ 290 300 310  
U68749 LALFVNFLQSYVLRGGKOKYKAVPKKKNN (F56H11.4) 260 270 280.

FIG. 39

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SCORES Init: 189 Init: 264 Opt: 358 Smith-Waterman score: 358; 28.7% identity in 296 aa overlap

MAE0 MAAAILDKVNFQGIDQPFGTQKLDTYFQAQYELVTKSIDSFVFOEGITPLSTQREYAMW-T 10 20 30 40 50 59  
U68749 MAQHPLVQRLDVKFDT...-KRFIAIAITHGPNNPDAEGRKFFADHFDVITQAS (F56H11.4) 10 20 30 40 50 50.

MAE0 ITYFWIFGGRQIMKSQDAFKLK -PLFILNFLLTIASGSLLLFENLYVPLTILARNGLFY 60 70 80 90 100 110  
U68749 ILYMWWFGTKWFRNRFQFLTIPLNW -NFIILAAFSITAGANKMPEFFGTIAKGVIA (F56H11.4) 60 70 80 90 100 110.

MAE0 AICDDGAMTORLELYYNYLW -KYNELADTVFLVLUKKPLFELHLYFHHSITMVLCFVQL 120 130 140 150 160 170  
U68749 SYCKVDFDTKGENGWYMLFMASKLFELVOTIFLVRKRPLHNYHHTLMTYAWSH (F56H11.4) 120 130 140 150 160 170.

MAE0 GGYTSISWAPITLNLTVHFMY -YYWRSAAVRL -WKKQYLTTLQIVQFVLDGFIY 180 190 200 210 220 230  
U68749 PLTPGFNRGYLYNFVHAFMYSYFLRSK -IRVPGFIAQITSLSQIVQFTISCAVLH (F56H11.4) 180 190 200 210 220.

MAE0 CAYT-YFAFTYFPMAPWVKGCACTEGALFCGILLSSYLLFINFRITY -...NAKAK 240 250 260 270 280  
U68749 LGYLMHFTNACDFEPVFLA -...V...-MDTTLALFVNFFLQSYVLRGGKOKYK (F56H11.4) 230 240 250 260 270 280.

MAE0 AAKEGSNNFTPKTVKSGGSPKKPSKSHI 290 300 310  
U68749 AVPKKKNN (F56H11.4)

FIG. 40

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SCORES Init1: 77 Initn: 155 Opt: 264  
 Smith-Waterman score: 264: 27.2% identity in 206 aa overlap

GLELO AALVAQAEKYYPTIVHTRGEVAVESPLAREPLMNPFLVILIVLAYLVTFGQHQM  
 PTRKINMDSITPNYSTIFTDFDFTQRTKWLLENWTFYFCGTYMLVIFGQHFMQ  
 DM1 10 20 30 40 50 60

100 110 120 130 140 150  
 NFERFEVKTFSLLHNFCLVISAYNCGGILYEAQAA-NYGLFENAAADHTF-KGGLPMAK  
 |:|::|:|::|:|::|:|::|:|::|:|::|:|::|:|::|:|::|:|::|:|::|:|::|:  
 NRPFRQLRGPFLITWNTLAAFTINGAARTAPELIVLRYGLFHSVCPYSFEQDRVCGF  
 DM1 70 80 90 100 110 120

160 170 180 190 200 210  
 MINLFYFSKIMEFVDTMINWKKRNQTSFELHVVHSSIFTIMULVTFVAPNGEAFSSA  
 |||:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:  
 WTWLFVLSKPELGDFTIFLRK-.QPLIFLHWYHHTVLIYSNF-SYTEYSSARWFVTV  
 DM1 130 140 150 160 170 180

220 230 240 250 260 270  
 LNSFTHVMMGYYFLSALGKQUSFINKYITRSQNTQFCMMSYQSSIDMYAMKVLRPGY  
 :|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:|:  
 MNYCVHSMSYALKAARRNPPRFISHIITSLQLAQMTIGCAINWANGFLKTHGTXSC  
 DM1 190 200 210 220 230 240

280 290 300 310  
 PFFITALLWFYMMTMLGLFNFYRKNAKLAQAKADAKEKARKLQ  
 HISORNINSLAMYSSYFVLFARFFYKAYLAPGGHKSRRMA  
 DM1 250 260 270 280

SCORES Init1: 181 Initn: 279 Opt: 328  
 Smith-Waterman score: 328: 30.0% identity in 237 aa overlap

GLELO VTKGKSDSFVQEGVITPLSTQREVAWNTTIVFVTFGGRQIMKSQDAFKLKLPLFLHNFL  
 IFDFENDFTQRTKWLLENWTFYFCGTYMLVIFGQHFMQNRPRFQLRQLPLIWTNL  
 DM1 30 40 50 60 70 80  

100 110 120 130 140 149  
 LTIASGLLLFIENLVPFLARNGLFLVAGCGDGAWTQ-RLELLY-YNYLWKNWELADTV  
 |:|::|:|::|:|::|:|::|:|::|:|::|:|::|:|::|:|::|:|::|:|:  
 LAMFSIMGAARTAPELIVLRYGLFHSVCPYSFEQDRVCGFWTWFLVSLKPELGDIT  
 DM1 90 100 110 120 130 140

150 160 170 180 190 200  
 ELVLLKKPFLFLHVFHHSITWLCFVQLGGVTS-VSNVPIITNLTHVVFVYYYMRSAAG  
 |:|::|:|::|:|::|:|::|:|::|:|::|:|::|:|::|:|::|:|:  
 FTVLRKQPLFLHMYHHTVLIYSWFSYETVTSARWF-IVVNYCHSVMSYVYALKAA  
 DM1 150 160 170 180 190 200

210 220 230 240 250 260  
 VRI-WMKQYLTTLQIVQFLVLDLGFYFCATTYFAFTYFPWAPNGKCATGEGAALFGCG  
 |:|::|:|::|:|::|:|::|:|::|:|::|:|:  
 FNPPRFISMITSLQLAQMIIG.....GAINWANGFLK-THGTXSCHISQRNINSLA  
 DM1 210 220 230 240 250

270 280 290 300 310  
 LSSYLLLFINFYRITWAKAKAAKERGSNFTPKTVKSGSPKPKSKSHI  
 |:|::|:|::|:|::|:|:  
 MYSSYFVLFARFFYKAYLAPGGHKSRRMA  
 DM1 260 270 280

FIG. 41

FIG. 42

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1 ATGAAACATT TTGATGGATC ACTTAGTACCA TATTCAAGG CATTGCTAGG  
 51 CCCTCGAGAT ACTAGAGTAA AAGGATGTT TCTTCTGGAC AATTATATAC  
 101 CCACATTAT CTCCTCTGTC ATATATTAC TAATTTGATG GCTGGGGACCA  
 151 AAAATACATGA GGATTAACAA GGCATTCTCT TGCGGGGGAA TTTTAGTGGT  
 201 GTATAACCTT GGAACACAC TGCTGTCTCT GTATATGTTCT TTGAGATTTAG  
 251 TAACAGGAGT ATGGAGGG AAATACACT TCTCTCTGCA GGGCACACGCG  
 301 ACCGGAGGAG AATCAGATAT GAAGATTAC TGTTCTCTCT GTGTTACTA  
 351 CTCTCTCAA CTCATAGAT TATGGACAC TTTCTCTCTC ATCCCTGCAGA  
 401 AGAACAAACCA CGAGATCAGG GTCTGCAAG TCTAACACCA TGCCCTGATG  
 451 CTGAAACATCT GTGGGGTTGT GATGAACTGG GTCCCTCTGG GGCACCTCTTA  
 501 TTTTGGTGGC ACACATTAATA GCTTCATCCA GCTTCCTCATG TACTCTTACT  
 551 ATGGTTTGTG GTCAGTCCTT TCCATGGTC CATAACCTCTG GTGGAGAAG  
 601 TACATCACTC AGGGCAGCT GCTTCAGTT GTGCTGACAA TCATCCAGAC  
 651 CAGCTGGGG GTCATCTGGC GTGTCAGATT CCCTCTGGT TGGTTGATT  
 701 TCCAGATTGG ATACATTATT TCCCTGATG CTCTCTTCAC AACTCTTAC  
 751 ATTCAGACCT ACACAGAAA AGGGGCTCC CGAGGAAG ACCACCTGAA  
 801 GGACCAACAG AATGGGTCTG TGGCTGCTG GAATGGACAC ACCAACAGT  
 851 TTTCACCCCT GAAAAACAT GTAAAGCCAA GGAGGCTGG GAAGGATTGA  
 901 AGTCAAAAGAA TTGA

FIG.43

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1 MEHFADSLST YFKALLGPRD TRVKGMFLD NYIPPFICSV YILLWILGP  
 51 KYMRNKOFS CRGLWVYNL GLTLLSLYMF CELVIGWEG KYNFFCGTR  
 101 TAGESDMKII RVLMYYFSK LIEFDOTFFF IIRKNNQIT VLHVYHASM  
 151 LNTIAFVNNW VPCGHSTFGA TLNSFHVLW YSYVGLSSVP SMRPLMWWK  
 201 YITQGQLQF VLTIIQTSRG VIWPCFTPLG WLYFQIGYI SLIALETFNFY  
 251 IOTYNNKGAS RRQDHLQHQ NSSVAANH TNSFSPLENN VKPRKLKD\*

FIG.44

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1 ATGGCTCAGC ATCCGCTGT TCAAGGGCTT CTGATGTCA AATTGACAC  
 51 GAAAGATT GTGGCTATT CTACTCATGG GCAAAAGAT TTCCCTGAGC  
 101 CAGAAGGTG CAAGGTTG CTGATGTTCTT GCTGATCACT TTGATGTTAC TATTAGGCT  
 151 TCAATCCCTGT ACATGGTGT TGTGTTGGAA ACAAAATGGT TCATGGTAA  
 201 TCGTCAACCA TTCAATTGA CTATTCACCA CAACATCTGG AATTTCATCC  
 251 TCGCGCATT TCCATCGCA GGAGCTGTC AATGACCCC AGAGTTCTT  
 301 GGACCATG CCACAAAGG ATTGTCGA TCTTACTGCA AAGGTTGAA  
 351 TTTCAAGAA GGAGGAAATG GATACTGGGT GGGGCTTC ATGGCTTCCA  
 401 AACTTTTGA ACTTTTGAC ACCATCTCT TGGTTCTCCG TAACGTCGA  
 451 CTCACTGTCCTTCACTGTGTCACCAATT CTACCATGA TCTACGCTG  
 501 GTACTCTCAT CCATGACCC CAGGATTCA CAGATACGGAA ATTATATTA  
 551 ACTTTGCTG CCAAGCCTTC ATGACTCTT ACTACTCTT TCCTCTGATG  
 601 AAGATTGCG TGCGAGGATT CATGCCCAA GCTATCACAT CTCTCAAT  
 651 CGTTCAATT ATCATCTCTT GCGCGGTCT TGTCTCATCTT GTTATCTCA  
 701 TGACATTCAC CAATGCCAAC TGTTGATTTCG AGCCATCAGT ATTCAAGTC  
 751 GCAGTTCA TGGCACAC ACATTTGGCT CTTTCGTCA ACTTCTTCC  
 801 CCAATCATAT GTTCTCCGG GAGGAAAGA CAAGTACAAG GCAGTGCCTA  
 851 AGAGAAGAA CAACTAA

FIG. 45

| Fatty Acid       | % TOTAL FATTY ACID | % TOTAL FATTY ACID | % TOTAL FATTY ACID         | % TOTAL FATTY ACID | % CONVERSION=PRODUCT/(SUBSTRATE+PRODUCT) |
|------------------|--------------------|--------------------|----------------------------|--------------------|--|
| HOST(PLASMID)    | 334(pYX242)        | 334(pRAE-58-A1)    | 334(pYX242)                | 334(pRAE-58-A1)    |  |
| AMOUNT SUBSTRATE | 25 $\mu$ M GLA     | 25 $\mu$ M GLA     | 25 $\mu$ M AA              | 25 $\mu$ M AA      |  |
|                  | 0.04               | 0.09               | (50.34%) <sup>a</sup> 2.75 | 0.03               | 0.02                                     |
| C18:3n-6         |                    |                    |                            |                    | ND (23.37%) <sup>a</sup> 1.21            |
| C20:4n-6         |                    |                    |                            |                    | 7.48 3.97                                |
| C20:3n-6         |                    |                    |                            |                    | 11.33 2.01                               |
| C18:1n-7         | 41.11              | 34.72              | 41.49                      | 35.07              | 11.57                                    |
| C20:1n-7         |                    | 0.04               | 11.33                      |                    | 1.62                                     |
| C18:1n-9         | 15.60              | 15.66              | 15.16                      | 14.57              | 0.06                                     |
| C20:1n-9         | 0.06               | 0.22               | 0.22                       | 0.23               | 0.06                                     |
| C18:1n-5         | 0.11               | 0.62               | 0.62                       | 0.58               | 0.12                                     |
| TOTAL UDP        |                    | 370                | 969                        | 359                | 514                                      |

FIG. 46

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1 MAQPLVQRL LDVKFDIKRF VAIATHGPKN FPDAEGRKFF ADHFDVITQA  
 51 SII YMWIVFG TKHFMNRQP FQLTPLNW NFTLAFTSIA GAKNRPFF  
 101 GTIANGTVA SYCKVDFTK GENGYWWLF MASKLFLVLD TIFLVRKRP  
 151 LMFLHMYHI LTMIAVYSH PLTPGPNRYG IYLNFWHAF WYSYFELRM  
 201 KIRPGFIAQ AITSLQIVQF IISCAVLAHL GYLMHFTNAN CDFEPSYFKL  
 251 AVFIDDTYLA LFNFQFQSY VLRGKOKYK AVPKKKIN

FIG.47

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| HOST(PLASMID)    | 334(pX242)       | 334(pRE-21)      | 334(pRE-22)      | FATTY ACID    | % TOTAL FATTY ACID   | % TOTAL FATTY ACID | % TOTAL FATTY ACID   | % TOTAL FATTY ACID | % CONVERSION=PRODUCT/(SUBSTRATE+PRODUCT) | TOtal LPD (mg) |       |
|------------------|------------------|------------------|------------------|---------------|----------------------|--------------------|----------------------|--------------------|--|----------------|-------|
|                  |                  |                  |                  | 50 $\mu$ M MA | 50 $\mu$ M MA        | 50 $\mu$ M MA      | 50 $\mu$ M MA        | 50 $\mu$ M MA      |  |                |       |
| ADDED SUBSTRATES | 50 $\mu$ M GLA + | 50 $\mu$ M GLA + | 50 $\mu$ M GLA + |               |                      |                    |                      |                    |  |                |       |
|                  | 0.09             | 0.18             | 0.13             | 9.9           | 9.22                 | 12.46              | 1.46                 | 1.46               | 0.13                                     | 0.02           | 0.02  |
| C16:0            |                  |                  |                  | 1.46          | 1.46                 | 2.41               | 2.41                 | 2.41               | 1.49                                     | 1.49           | 1.49  |
| C16:1            |                  |                  |                  | 0.09          | 0.09                 | 0.18               | 0.18                 | 0.18               | 0.13                                     | 0.13           | 0.13  |
| C18:0            |                  |                  |                  | 9.9           | 9.9                  | 12.46              | 12.46                | 12.46              | 9.9                                      | 9.9            | 9.9   |
| C18:1n-9         |                  |                  |                  | 1.46          | 1.46                 | 2.41               | 2.41                 | 2.41               | 1.49                                     | 1.49           | 1.49  |
| C18:3n-6         |                  |                  |                  | 4.03          | 4.03                 | 4.92               | 4.92                 | 4.92               | 3.91                                     | 3.91           | 3.91  |
| C20:3n-6         |                  |                  |                  | 10.02         | 10.02                | 11.89              | 11.89                | 11.89              | 8.69                                     | 8.69           | 8.69  |
| C20:4n-6         |                  |                  |                  | 46.98         | 46.98                | 28.87              | 28.87                | 28.87              | 35.25                                    | 35.25          | 35.25 |
| C20:4n-6         |                  |                  |                  | 0.13          | (11.1%) <sup>a</sup> | 1.48               | (19.4%) <sup>a</sup> | 2.09               | 0  | 0              | 0     |
|                  |                  |                  |                  |               |                      |                    |                      |                    |  | 174            | 187   |
|                  |                  |                  |                  |               |                      |                    |                      |                    |  |                |       |

FIG.48

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1 ATGAACATGT CAGGTGAC TTTACAGAA TATGATTG AAAAGCAGTT  
 51 CAACGAGAAT GAAGGCCATCC AATGGATGCG GAAAACCTGG AAGAAATCTT  
 101 TCCGTTC TGCCTGTAT GCTGCCTTA TATTCGGTGG TCGGACCTA  
 151 ATGATAAAC GAGCAAAAGTT TGAACGTAGG AAGCCATTAG TGCTCTGGC  
 201 TCTGACCCCTT GCGAGCTCTCA GTATATTCCG TGCTCTTCGA ACTGGTGCT  
 251 ATATGGTGTAA CATTGGATG ACCAAAGGCC TGAGGAGTC AGTTGGAC  
 301 CAGGGTTTTT ACAATGGACC TGTACGAAA TTCTGGCCTT ATGCAATTGT  
 351 GCTAAAGCAA GCACCGAAC GAGGAGATC AATATTGATT ATTCTGAGA  
 401 AGCGAAAGT GATCCTTCCTG CACTGGTAC ACCACATGAC TGTGGTCCCTG  
 451 TACTCTTGT ACTCCCTACAA AGACATGGTT GCGGGGGAG GTTGGTCAAT  
 501 GACTATGAC TATGGGGTGC AGCCGTGAT GTACTCTTAC TATGCCCTGC  
 551 GGGGGCAGG TTTCCGAGTC TCCCGGAAGT TTGGCATGT CATACCTG  
 601 TCCAGATCA CTCAGATGCT GATGGGGCTGT GTGGTTAACT ACCTGGCTT  
 651 CTGTGGATG CAGGATGACG AGTGTCACTC TCACCTTCAG AACATCTCT  
 701 GGTCCCTACT CATGTAACCTC AGCTACCTG TGCTCTCTG CCATTTCTC  
 751 TTGGAGGCT ACATGGCAA AATGAGAAA ACAACGAAAG CTGAATAG

FIG.49

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1 MNNSVLTLQE YEEFKQFNEN EAQNMQENW KISFLSALY AAFIFGGRHL  
 51 MNIRRAKFEFL KPLVLMSLTL AVFSIFGALR TGAYWVILM TRGLKQSVCD  
 101 QGFYNGPVSX FMAYAFVLSK APELGDITFI ILRKQKLIFL HMYHHITVLL  
 151 YSWYSKDMW AGGGWFMNTIN YGVHAWVSY YALRAAGFRV SRKFAMFTTL  
 201 SQITQMLMGC VINYLFCM QHDOCHSHFQ NTWNSMML SYLVLFCHFF  
 251 FEAYIGKMRK TTAE\*

FIG.50

FIG. 52

$$\text{NO} = \text{NOT DETECTED}$$

SUBSTITUTE SHEET (RULE 26)

FIG. 51

$$(\%) \text{ CONVERSION} = \text{PRODUCT} / (\text{SUBSTRATE} + \text{PRODUCT})$$

SUBSTITUTE SHEET (RULE 26)

FIG. 53A

**SUBSTITUTE SHEET (RULE 26)**

(%CONVERSION)=PRODUCT/(ND = NOT DETECTED)

FIG. 53B

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FIG. 53C

(% CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT)

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FIG. 54

|     |             |            |             |            |            |
|-----|-------------|------------|-------------|------------|------------|
| 1   | ATGGACAGC   | TGAAGCCCTT | TGATAATGAA  | GTCATACTT  | TCTGGACAA  |
| 51  | CATGTTGGA   | CCACGAGATT | CTCGAGTTG   | CGGTGGTTC  | CTGCTGGACT |
| 101 | CTTACCTTC   | CACCTTCATC | CTTACCATCA  | CTGACTCTCT | CTGATATGG  |
| 151 | CTGGTAAAC   | AGTACATGAA | GAACAGGCC   | GTCTGTCTC  | TCAGGGCAT  |
| 201 | CTTACCTTG   | TATACCTCG  | CAATCACACT  | TCTTCTCG   | TATATGCTGG |
| 251 | TGAGCTCA    | CTCTCACAG  | TGGGAGGGG   | GTAAACATT  | GCAGCTTCAG |
| 301 | AATCTGACA   | GTGAGGAGAA | AGTGTAGTC   | GGGTAGGCA  | AGGCTTGTG  |
| 351 | GTGGTACTAC  | TTCCTCAAC  | TAGTGGATG   | CTGGACACG  | ATTTCTTTC  |
| 401 | TCTTACGAA   | AAAGACCCAT | CAGATCACCT  | TCTTCATGT  | CTATCACCC  |
| 451 | GGGTCACTG   | TCAACATCTG | GTTGGTGTGTT | TGAACATGGA | TACCTTGTGG |
| 501 | TCAAAGCTTC  | TTTGACCCA  | CCCTGACAG   | CTTATCCAC  | ATTCCTATGT |
| 551 | ACTCTCTACTA | CGGCCCTGTC | GTGTTCCCGT  | CCATGCCAA  | GTACCTTGG  |
| 601 | TGAAAGAAGT  | ACCTCACACA | GGCTCACGTC  | GTGAGTTTG  | TACTCACCCT |
| 651 | CACGACAGC   | CTGAGTGC   | TGGTAGAGCC  | CTCTGGCTTC | CCCTTTGGCT |
| 701 | GTCTCATCTT  | TCTACATCA  | TATATGATGAA | CGCTGGTCA  | CTCTGTCCTA |
| 751 | AACCTCTTCA  | TTCAGACATA | CCGGAAAAAA  | CCATGTGAGA | AAGGTGTCGA |
| 801 | AGAGAAAGA   | GTGAGAGATG | GTTCCCCCAA  | AGCCCCACTA | ATTGTGCTTA |
| 851 | ATGGATGAC   | GGAAAGAG   | GTCTAAATA   |            |            |

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1 MEQLKAFDNE WNAELDNMFG PROSRVGF LLDSYLPFII LITTYLLSIW  
 51 LGNKYMKNRP ALSRGTILTI YNLATLSSA YMLVETLSS WEGGYNLQQ  
 101 NLDSEAGEGDV RVAKVLMAMY FSKLVEFLDT IFFVLRKTN QITFLVYHH  
 151 ASMFNIWCV LNWPCCGSF FGPTLNSFTH TLMYSYGLS VFPShKYLW  
 201 WKKYLTOAQL VQFLTITH LSAWKPCGF PFGCLIFQSS YMTTIVLFL  
 251 NYTQTYRKK PVKKELOKEE VKNGFKPAHL IVANGNTDKK AQ\*

FIG.55

|               | 208      | 126      | 115      | 189      | 158      | 149      | 124      | 433      | 221      | 21       | 127      | 126      |
|---------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| TOTAL UPD     | ND       |
| C22-5h-3      | ND       |
| C22-4h-6      | ND       |
| C22-3h-3      | ND       |
| C22-4h-3      | ND       |
| C22-4h-6      | ND       |
| C20-5h-3      | ND       |
| C20-4h-6      | ND       |
| C20-4h-6      | 0.10     | 0.77     | ND       |
| C20-4h-7      | ND       |
| C18-4h-3      | ND       |
| C18-4h-6      | 1.25     | 1.21     | ND       |
| C18-4h-7      | 1.25     | 1.20     | 1.57     | 1.10     | 1.50     | 1.13     | 1.18     | 1.48     | 1.72     | 1.59     | 1.33     | 1.45     |
| C18-4h-9      | 1.25     | 1.16     | 1.23     | 1.17     | 1.17     | 1.13     | 1.18     | 1.19     | 1.38     | 1.33     | 1.37     | 1.38     |
| CONCENTRATION | 25uM     |
| SUBSTRATE     | GLA      |
| PASSED        | PRM-E-84 |
|               | 334      | 334      | 334      | 334      | 334      | 334      | 334      | 334      | 334      | 334      | 334      | 334      |

FIG.56

(\*) CONVERSION=PRODUCT/(SUBSTRATE+PRODUCT)  
 ND = NOT DETECTED



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FIG. 57C

(% CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT)  
ND = NOT DETECTED

| HOST      | 334         | 334        | 334         | 334        | 334        | 334        | 334         | 334         | 362         | 384        | 173        | 393        | 124        | 280        | 137        | 151        | 190        | 200           |
|-----------|-------------|------------|-------------|------------|------------|------------|-------------|-------------|-------------|------------|------------|------------|------------|------------|------------|------------|------------|---------------|
| SUBSTRATE | PLA-E-84    | PLX242     | PLA-E-84    | PLX242     | PLA-E-84   | PLA-E-84   | PLA-E-84    | PLA-E-84    | 25μM        | 25μM       | 25μM       | 25μM       | 25μM       | 25μM       | 25μM       | 25μM       | 25μM       | % TOTAL LIPI  |
| PLASMID   | PLX242      | PLA-E-84   | PLX242      | PLA-E-84   | PLX242     | PLA-E-84   | PLA-E-84    | PLA-E-84    | 25μM        | 25μM       | 25μM       | 25μM       | 25μM       | 25μM       | 25μM       | 25μM       | 25μM       | CONCENTRATION |
| C18:1-n-9 | 16.69       | 16.38      | 18.24       | 15.95      | 14.07      | 15.16      | 16.05       | 15.06       | 17.47       | 17.47      | 17.15      | 17.15      | 17.15      | 17.15      | 17.15      | 17.15      | 17.15      | C18:2-n-6     |
| C18:1-n-7 | 1.37        | 1.43       | 1.71        | 1.40       | 1.37       | 1.47       | 1.67        | 1.51        | 1.71        | 1.71       | 1.75       | 1.75       | 1.75       | 1.75       | 1.75       | 1.75       | 1.75       | C18:3-n-7     |
| C18:3-n-6 | 0.08        | 0.08       | 0.12        | 0.04       | 0.13       | 0.06       | 0.11        | 0.18        | 0.13        | 0.12       | 0.12       | 0.12       | 0.12       | 0.12       | 0.12       | 0.12       | 0.12       | C18:4-n-3     |
| C18:3-n-3 | (1.32)0.06  | (3.62)0.16 | ND          | 2.28       | 2.39       | ND         | ND          | ND          | ND          | ND         | ND         | ND         | ND         | ND         | ND         | ND         | ND         | C20:3-n-3     |
| C18:4-n-3 | ND          | ND         | ND          | ND         | ND         | ND         | ND          | ND          | ND          | ND         | ND         | ND         | ND         | ND         | ND         | ND         | ND         | C20:4-n-3     |
| C20:3-n-3 | (1.32)0.06  | (3.62)0.16 | ND          | 2.28       | 2.39       | ND         | ND          | ND          | ND          | ND         | ND         | ND         | ND         | ND         | ND         | ND         | ND         | C22:3-n-3     |
| C20:4-n-3 | ND          | ND         | ND          | ND         | ND         | ND         | ND          | ND          | ND          | ND         | ND         | ND         | ND         | ND         | ND         | ND         | ND         | C22:5-n-6     |
| C22:5-n-3 | ND          | ND         | ND          | ND         | ND         | ND         | ND          | ND          | ND          | ND         | ND         | ND         | ND         | ND         | ND         | ND         | ND         | C22:5-n-3     |
| C22:4-n-3 | ND          | ND         | ND          | ND         | ND         | ND         | ND          | ND          | ND          | ND         | ND         | ND         | ND         | ND         | ND         | ND         | ND         | C24:3-n-3     |
| C24:3-n-3 | 0.65        | 0.43       | 1.41        | 0.58       | ND         | ND         | ND          | ND          | ND          | ND         | ND         | ND         | ND         | ND         | ND         | ND         | ND         | C24:3-n-3     |
| C24:3-n-6 | ND          | ND         | ND          | ND         | ND         | ND         | ND          | ND          | ND          | ND         | ND         | ND         | ND         | ND         | ND         | ND         | ND         | C24:3-n-6     |
| C24:3-n-7 | 0.64        | 0.64       | 0.64        | 0.64       | 0.64       | 0.64       | 0.64        | 0.64        | 0.64        | 0.64       | 0.64       | 0.64       | 0.64       | 0.64       | 0.64       | 0.64       | 0.64       | C24:3-n-7     |
| 1         | ATGGAACATT  | TGGATGCGTC | ACTGAATGACC | TATTTCAAGG | CCTTCCTGG  | CCCCGGAGAT | ACAGAAGTCA  | AAGGATGTT   | CCTCTGGAC   | AATTACATCC | CTAGGTTGT  | CTGTTCTGTT | ATTACTTAC  | CTATGTTCT  | TGCGAGGGA  | TGTCAGTT   | TATGAGTTGG | ATGAAACCTT    |
| 51        | CTGAGCTGT   | GTGGAGGGC  | AAATACACT   | TTTTCGCA   | GGGACAGCC  | AGGGGGAG   | ACAGAAGTCA  | AAGGATGTT   | CCTCTGGAC   | AATTACATCC | CTAGGTTGT  | CTTCTCTTC  | ATCCCTCGCA | CTATGTTCT  | TGCGAGGGA  | TGTCAGTT   | TATGAGTTGG | ATGAAACCTT    |
| 101       | AAATACATGA  | AGAACCGGGA | GGCGTCTCT   | TGCGAGGGA  | TGTCAGTT   | CTTCTCAA   | CTCATCGAT   | TCAGGACAC   | CTTCTCTTC   | ATCCCTCGCA | CTTCTCTTC  | ATCCCTCGCA | CTATGTTCT  | TGCGAGGGA  | TGTCAGTT   | TATGAGTTGG | ATGAAACCTT |               |
| 151       | GTATACCTT   | GGACTCACCC | TGCTGTCT    | CTATGTTCT  | TGCGAGGGA  | CTTCTCAA   | CTCATCGAT   | TCAGGACAC   | CTTCTCTTC   | ATCCCTCGCA | CTTCTCTTC  | ATCCCTCGCA | CTATGTTCT  | TGCGAGGGA  | TGTCAGTT   | TATGAGTTGG | ATGAAACCTT |               |
| 201       | CTGAGCTGT   | GTGGAGGGC  | AAATACACT   | TTTTCGCA   | GGGACAGCC  | AGGGGGAG   | ACAGAAGTCA  | AAGGATGTT   | CCTCTGGAC   | AATTACATCC | CTAGGTTGT  | CTTCTCTTC  | ATCCCTCGCA | CTATGTTCT  | TGCGAGGGA  | TGTCAGTT   | TATGAGTTGG | ATGAAACCTT    |
| 251       | TGACAGCTGT  | GTGGAGGGC  | AAATACACT   | TTTTCGCA   | GGGACAGCC  | AGGGGGAG   | ACAGAAGTCA  | AAGGATGTT   | CCTCTGGAC   | AATTACATCC | CTAGGTTGT  | CTTCTCTTC  | ATCCCTCGCA | CTATGTTCT  | TGCGAGGGA  | TGTCAGTT   | TATGAGTTGG | ATGAAACCTT    |
| 301       | AGGGGGAG    | ACAGAAGTCA | AAGGATGTT   | CTAGGATCAT | GGAGATCATC | CGCGTCTCT  | GGTGTACTA   | CTTCTCAA    | CTCATCGAT   | TCAGGACAC  | CTTCTCTTC  | ATCCCTCGCA | CTATGTTCT  | TGCGAGGGA  | TGTCAGTT   | TATGAGTTGG | ATGAAACCTT |               |
| 351       | CTTCTCAA    | CTCATCGAT  | TCAGGACAC   | CTTCTCTTC  | ATCCCTCGCA | CTATGTTCT  | TGCGAGGGA   | TGTCAGTT    | TATGAGTTGG  | ATGAAACCTT | CTGAGCTGT  | GTGGAGGGC  | AAATACACT  | TGCGAGGGA  | TGTCAGTT   | TATGAGTTGG | ATGAAACCTT |               |
| 401       | AGAACACCA   | GGAGATCATC | GGAGATCATC  | GGAGATCATC | GGAGATCATC | GGAGATCATC | GGAGATCATC  | GGAGATCATC  | GGAGATCATC  | GGAGATCATC | GGAGATCATC | GGAGATCATC | GGAGATCATC | GGAGATCATC | GGAGATCATC | GGAGATCATC | GGAGATCATC |               |
| 451       | CTAACACATCT | GTGGAGGGC  | AAATACACT   | TTTTCGCA   | GGGACAGCC  | AGGGGGAG   | ACAGAAGTCA  | AAGGATGTT   | CCTCTGGAC   | AATTACATCC | CTAGGTTGT  | CTTCTCTTC  | ATCCCTCGCA | CTATGTTCT  | TGCGAGGGA  | TGTCAGTT   | TATGAGTTGG | ATGAAACCTT    |
| 501       | TTTGGTGGG   | ACACTCAACA | GCTTATCCCA  | TGTCAGTT   | TGTCAGTT   | CTCGATCCCG | TCCATGTCGTC | CTCAACCTCTG | GTGGAGGAAAG | AAACTCTAC  | ATGAGCTGT  | GTGGAGGGC  | AAATACACT  | TGCGAGGGA  | TGTCAGTT   | TATGAGTTGG | ATGAAACCTT |               |
| 551       | ATGAGCTGT   | GTGGAGGGC  | AAATACACT   | TGCGAGGGA  | TGTCAGTT   | CTCGATCCCG | TCCATGTCGTC | CTCAACCTCTG | GTGGAGGAAAG | AAACTCTAC  | ATGAGCTGT  | GTGGAGGGC  | AAATACACT  | TGCGAGGGA  | TGTCAGTT   | TATGAGTTGG | ATGAAACCTT |               |
| 601       | TAATACACTC  | AGGGGGAG   | GGTGTACTA   | CTTCTCAA   | CTCATCGAT  | TCAGGACAC  | CTTCTCTTC   | ATCCCTCGCA  | CTATGTTCT   | TGCGAGGGA  | TGTCAGTT   | TATGAGTTGG | ATGAAACCTT | CTGAGCTGT  | GTGGAGGGC  | AAATACACT  | TGCGAGGGA  | ATGAAACCTT    |
| 651       | GACTCGGGG   | GTGGAGGGC  | AAATACACT   | TGCGAGGGA  | TGTCAGTT   | CTCGATCCCT | CCCTCTCGGG  | GGGACAGCC   | AGGGGGAG    | ACAGAAGTCA | AAGGATGTT  | CTCAGATTGG | TCCCTGATTG | CTCTCTTCAC | AAACTCTAC  | ATGAGCTGT  | GTGGAGGGC  | ATGAAACCTT    |
| 701       | TCAGATTGG   | ATACATGATT | TCCCTGATTG  | CTCTCTTCAC | AAACTCTAC  | CTCGATCCCT | CCCTCTCGGG  | GGGACAGCC   | AGGGGGAG    | ACAGAAGTCA | AAGGATGTT  | CTCAGATTGG | TCCCTGATTG | CTCTCTTCAC | AAACTCTAC  | ATGAGCTGT  | GTGGAGGGC  | ATGAAACCTT    |
| 751       | ATTAGACTT   | ACAAACAGA  | AGGGGGAG    | GGGACAGCC  | AGGGGGAG   | GGGACAGCC  | AGGGGGAG    | GGGACAGCC   | AGGGGGAG    | GGGACAGCC  | AGGGGGAG   | GGGACAGCC  | AGGGGGAG   | GGGACAGCC  | AGGGGGAG   | GGGACAGCC  | AGGGGGAG   | GGGACAGCC     |
| 801       | GGCCACCA    | AGGGGGAG   | GGGACAGCC   | AGGGGGAG   | GGGACAGCC  | AGGGGGAG   | GGGACAGCC   | AGGGGGAG    | GGGACAGCC   | AGGGGGAG   | GGGACAGCC  | AGGGGGAG   | GGGACAGCC  | AGGGGGAG   | GGGACAGCC  | AGGGGGAG   | GGGACAGCC  | AGGGGGAG      |
| 851       | TCCCTCCCT   | GGAAACAGC  | GTGAGGCCA   | GGGACAGCC  | AGGGGGAG   | GGGACAGCC  | AGGGGGAG    | GGGACAGCC   | AGGGGGAG    | GGGACAGCC  | AGGGGGAG   | GGGACAGCC  | AGGGGGAG   | GGGACAGCC  | AGGGGGAG   | GGGACAGCC  | AGGGGGAG   | GGGACAGCC     |

FIG. 58

|   |  |    |   |
|---|--|----|---|
| 1 | MEHDASLT YKAFLGPRD TRKGWFLDO NYIPFTFCV SYLLIWLG    | 51 | KYMKARQPF5 CRGILQYLN GLTLLSYMF YELVGTMEG KYNFFCGTR    |
| 1 | SAGESDK11 RVLWNYFSK LIEFDTFFF ILRBNHQHIT VLHVYHATM | 51 | LNIAWFMWV VPGHSYFGA TLNSEIHWL YSYGLSSIP SMRPLWKK      |
| 1 | YITQGLVQF VLTIIOTG5 VWPQSFPLG WLFFQIGMI SLIAFTNFY  | 51 | TOTYNGKGAS BRDQH KGHO NSVIAAVNGH TNSPERSIENS VPKRKKD* |
| 1 |  | 51 |   |

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09.61

$$(\% \text{ COMBUSTION}) = \text{PRODUCT} / (\text{SUBSTRATE} + \text{PRODUCT})$$

SUBSTITUTE SHEET (RULE 26)

**SUBSTITUTE SHEET (RULE 26)**

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FIG. 61

| HOST(PLASMID) | % TOTAL LPID |               |             |               |             |              | *INDICATES SUBSTRATE ADDED            |
|---------------|--------------|---------------|-------------|---------------|-------------|--------------|---------------------------------------|
|               | 334(PYX242)  | 334(PRET-22)  | 334(PYX242) | 334(PRET-22)  | 334(PYX242) | 334(PRET-22) |                                       |
| C18.0         | 17.4         | 17.4          | 26.62       | 13.01         | 40.1        | 47.67        | 5.03                                  |
| C18.1-n-7     | 5.79         | 4.94          | 3.62        | 2             | 4.86        | 1.18         | (12.54%) 1.18 (3.6%) 1.5 (7.53%) 3.88 |
| C18.1-n-7     | (3.9%) 0.85  | (9.12%) 1.78  | (3.5%) 0.97 | (12.54%) 1.18 | (3.6%) 1.5  | 1.18         | (1.1%) 0.3 (38.2%) 13.61              |
| C18.1-n-6     | 8.46         | 7.45          | 7.45        | 10.27         | 5.36        | 13.7         | 16.93                                 |
| C18.1-n-6     | *26.62       | *22.03        | 0.03        | 0.01          |             |              |                                       |
| C18.1-n-6     | (1.1%) 0.3   | (38.2%) 13.61 |             |               |             |              |                                       |
| TOTAL LPID    | 36           | 42            | 85          | 280           | 55          | 79           |                                       |
|               |              |               |             |               |             |              |                                       |

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FIG. 62A

| HOST(PLASMID)   | % TOTAL LPID |             |             |             |             |             | *INDICATES SUBSTRATE ADDED |
|-----------------|--------------|-------------|-------------|-------------|-------------|-------------|----------------------------|
|                 | 334(PRET22)  | 334(PRET22) | 334(PRET22) | 334(PRET22) | 334(PRET22) | 334(PRET22) |                            |
| C16.0           | 12.9         | 12.54       | 15.23       | 9.1         | 10.2        | 3.42        |                            |
| C16.1           | 37.71        | 23.83       | 24.87       | 16.61       | 18.375      | 7.66        |                            |
| C18.0           | 11.44        | 4.7         | 4.49        | 2.7         | 2.9         | 1.23        |                            |
| C18.1-n-9       | 14.03        | *16.87      | 9.54        | 6.74        | 6.39        | 2.99        |                            |
| C18.2-n-6       |              |             |             | 16.87       | 0.15        | 0.28        |                            |
| C18.3-n-6       |              |             |             |             | 0.05        |             |                            |
| C20.2-n-6       |              |             |             |             | 0.34        | *25.78      | 0.26                       |
| C20.3-n-6       |              |             |             |             |             |             | *75.72                     |
| C20.4-n-6       |              |             |             |             |             |             |                            |
| TOTAL LPID (mg) | 63           | 103         | 71          | 110         | 97          | 277         |                            |

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| HOST(PLASMID)          | 334(pRET-22+pcGR-4) | 334(pYX242+pcES2) |
|------------------------|---------------------|-------------------|
| ADDED SUBSTRATE        | 50 $\mu$ M GLA      | 50 $\mu$ M GLA    |
| FATTY ACID             | % TOTAL LIPID       |                   |
| C16:0                  | 15.92               | 15.07             |
| C16:1n-7               | 24.97               | 19.48             |
| C18:0                  | 8.52                | 6.48              |
| C18:1n-7               | 3.9                 | 1.61              |
| C18:1n-9               | 18.48               | 12.71             |
| C18:3n-6               | *7.0                | *10.54            |
| C20:0                  | 0                   | 0                 |
| C20:3n-6               | (27.81%) 4.36       | (1.58%) 0.17      |
| C20:4n-6               | (27.55%) 4.32       | 0                 |
| TOTAL LIPID ( $\mu$ g) | 508                 | 168               |

FIG. 63A

| HOST(PLASMID)          | 334(pRET-22+pcGR-4) | 334(pYX242+pcES2) |
|------------------------|---------------------|-------------------|
| ADDED SUBSTRATE        | 50 $\mu$ M STA      | 50 $\mu$ M STA    |
| FATTY ACID             | % TOTAL LIPID       |                   |
| C16:0                  | 18.74               | 16.21             |
| C16:1n-7               | 21.35               | 26.09             |
| C18:0                  | 6.78                | 7.57              |
| C18:1n-7               | 1.97                | 1.7               |
| C18:1n-9               | 20.73               | 22.41             |
| C18:3n-3               | *6.05               | *13.43            |
| C18:4n-3               | 0                   | 0.45              |
| C20:3n-3               | (15.88%) 1.68       | (4.73%) 0.69      |
| C20:5n-3               | (26.93%) 2.65       | (3.22%) 0.47      |
| TOTAL LIPID ( $\mu$ g) | 335                 | 161               |

\*INDICATES SUBSTRATE ADDED  
(% CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT)

FIG. 63B

| FATTY ACID    | % TOTAL LIPID | TOTAL LIPID ( $\mu$ g) |             |             |
|---------------|---------------|------------------------|-------------|-------------|
| HOST(PLASMID) | 334(pRET22)   | 334(pRET22)            | 334(pRET22) | 334(pRET22) |
| C16:0         | 13.91         | 15.06                  | 16.92       | 20.08       |
| C16:1         | 14.74         | 31.77                  | 23.57       | 20.17       |
| C18:0         | 4.06          | *4.85                  | 4.94        | 6.02        |
| C18:1n-9      | 6.65          | 13.59                  | 10.46       | 9.29        |
| C18:3m-3      | *38.66        |                        |             |             |
| C18:4n-3      |               |                        |             |             |
| C20:4n-3      |               |                        |             |             |
| C20:5n-3      |               |                        |             |             |
| C22:5n-3      |               |                        |             |             |
| C22:6n-3      |               |                        |             |             |
| C22:7n-3      |               |                        |             |             |
| C22:8n-3      |               |                        |             |             |
| C22:9n-3      |               |                        |             |             |
| C22:10n-3     |               |                        |             |             |
| C22:11n-3     |               |                        |             |             |
| C22:12n-3     |               |                        |             |             |
| C22:13n-3     |               |                        |             |             |
| C22:14n-3     |               |                        |             |             |
| C22:15n-3     |               |                        |             |             |
| C22:16n-3     |               |                        |             |             |
| C22:17n-3     |               |                        |             |             |
| C22:18n-3     |               |                        |             |             |
| C22:19n-3     |               |                        |             |             |
| C22:20n-3     |               |                        |             |             |
| C22:21n-3     |               |                        |             |             |
| C22:22n-3     |               |                        |             |             |
| C22:23n-3     |               |                        |             |             |
| C22:24n-3     |               |                        |             |             |
| C22:25n-3     |               |                        |             |             |
| C22:26n-3     |               |                        |             |             |
| C22:27n-3     |               |                        |             |             |
| C22:28n-3     |               |                        |             |             |
| C22:29n-3     |               |                        |             |             |
| C22:30n-3     |               |                        |             |             |
| C22:31n-3     |               |                        |             |             |
| C22:32n-3     |               |                        |             |             |
| C22:33n-3     |               |                        |             |             |
| C22:34n-3     |               |                        |             |             |
| C22:35n-3     |               |                        |             |             |
| C22:36n-3     |               |                        |             |             |
| C22:37n-3     |               |                        |             |             |
| C22:38n-3     |               |                        |             |             |
| C22:39n-3     |               |                        |             |             |
| C22:40n-3     |               |                        |             |             |
| C22:41n-3     |               |                        |             |             |
| C22:42n-3     |               |                        |             |             |
| C22:43n-3     |               |                        |             |             |
| C22:44n-3     |               |                        |             |             |
| C22:45n-3     |               |                        |             |             |
| C22:46n-3     |               |                        |             |             |
| C22:47n-3     |               |                        |             |             |
| C22:48n-3     |               |                        |             |             |
| C22:49n-3     |               |                        |             |             |
| C22:50n-3     |               |                        |             |             |
| C22:51n-3     |               |                        |             |             |
| C22:52n-3     |               |                        |             |             |
| C22:53n-3     |               |                        |             |             |
| C22:54n-3     |               |                        |             |             |
| C22:55n-3     |               |                        |             |             |
| C22:56n-3     |               |                        |             |             |
| C22:57n-3     |               |                        |             |             |
| C22:58n-3     |               |                        |             |             |
| C22:59n-3     |               |                        |             |             |
| C22:60n-3     |               |                        |             |             |
| C22:61n-3     |               |                        |             |             |
| C22:62n-3     |               |                        |             |             |
| C22:63n-3     |               |                        |             |             |
| C22:64n-3     |               |                        |             |             |
| C22:65n-3     |               |                        |             |             |
| C22:66n-3     |               |                        |             |             |
| C22:67n-3     |               |                        |             |             |
| C22:68n-3     |               |                        |             |             |
| C22:69n-3     |               |                        |             |             |
| C22:70n-3     |               |                        |             |             |
| C22:71n-3     |               |                        |             |             |
| C22:72n-3     |               |                        |             |             |
| C22:73n-3     |               |                        |             |             |
| C22:74n-3     |               |                        |             |             |
| C22:75n-3     |               |                        |             |             |
| C22:76n-3     |               |                        |             |             |
| C22:77n-3     |               |                        |             |             |
| C22:78n-3     |               |                        |             |             |
| C22:79n-3     |               |                        |             |             |
| C22:80n-3     |               |                        |             |             |
| C22:81n-3     |               |                        |             |             |
| C22:82n-3     |               |                        |             |             |
| C22:83n-3     |               |                        |             |             |
| C22:84n-3     |               |                        |             |             |
| C22:85n-3     |               |                        |             |             |
| C22:86n-3     |               |                        |             |             |
| C22:87n-3     |               |                        |             |             |
| C22:88n-3     |               |                        |             |             |
| C22:89n-3     |               |                        |             |             |
| C22:90n-3     |               |                        |             |             |
| C22:91n-3     |               |                        |             |             |
| C22:92n-3     |               |                        |             |             |
| C22:93n-3     |               |                        |             |             |
| C22:94n-3     |               |                        |             |             |
| C22:95n-3     |               |                        |             |             |
| C22:96n-3     |               |                        |             |             |
| C22:97n-3     |               |                        |             |             |
| C22:98n-3     |               |                        |             |             |
| C22:99n-3     |               |                        |             |             |
| C22:100n-3    |               |                        |             |             |
| C22:101n-3    |               |                        |             |             |
| C22:102n-3    |               |                        |             |             |
| C22:103n-3    |               |                        |             |             |
| C22:104n-3    |               |                        |             |             |
| C22:105n-3    |               |                        |             |             |
| C22:106n-3    |               |                        |             |             |
| C22:107n-3    |               |                        |             |             |
| C22:108n-3    |               |                        |             |             |
| C22:109n-3    |               |                        |             |             |
| C22:110n-3    |               |                        |             |             |
| C22:111n-3    |               |                        |             |             |
| C22:112n-3    |               |                        |             |             |
| C22:113n-3    |               |                        |             |             |
| C22:114n-3    |               |                        |             |             |
| C22:115n-3    |               |                        |             |             |
| C22:116n-3    |               |                        |             |             |
| C22:117n-3    |               |                        |             |             |
| C22:118n-3    |               |                        |             |             |
| C22:119n-3    |               |                        |             |             |
| C22:120n-3    |               |                        |             |             |
| C22:121n-3    |               |                        |             |             |
| C22:122n-3    |               |                        |             |             |
| C22:123n-3    |               |                        |             |             |
| C22:124n-3    |               |                        |             |             |
| C22:125n-3    |               |                        |             |             |
| C22:126n-3    |               |                        |             |             |
| C22:127n-3    |               |                        |             |             |
| C22:128n-3    |               |                        |             |             |
| C22:129n-3    |               |                        |             |             |
| C22:130n-3    |               |                        |             |             |
| C22:131n-3    |               |                        |             |             |
| C22:132n-3    |               |                        |             |             |
| C22:133n-3    |               |                        |             |             |
| C22:134n-3    |               |                        |             |             |
| C22:135n-3    |               |                        |             |             |
| C22:136n-3    |               |                        |             |             |
| C22:137n-3    |               |                        |             |             |
| C22:138n-3    |               |                        |             |             |
| C22:139n-3    |               |                        |             |             |
| C22:140n-3    |               |                        |             |             |
| C22:141n-3    |               |                        |             |             |
| C22:142n-3    |               |                        |             |             |
| C22:143n-3    |               |                        |             |             |
| C22:144n-3    |               |                        |             |             |
| C22:145n-3    |               |                        |             |             |
| C22:146n-3    |               |                        |             |             |
| C22:147n-3    |               |                        |             |             |
| C22:148n-3    |               |                        |             |             |
| C22:149n-3    |               |                        |             |             |
| C22:150n-3    |               |                        |             |             |
| C22:151n-3    |               |                        |             |             |
| C22:152n-3    |               |                        |             |             |
| C22:153n-3    |               |                        |             |             |
| C22:154n-3    |               |                        |             |             |
| C22:155n-3    |               |                        |             |             |
| C22:156n-3    |               |                        |             |             |
| C22:157n-3    |               |                        |             |             |
| C22:158n-3    |               |                        |             |             |
| C22:159n-3    |               |                        |             |             |
| C22:160n-3    |               |                        |             |             |
| C22:161n-3    |               |                        |             |             |
| C22:162n-3    |               |                        |             |             |
| C22:163n-3    |               |                        |             |             |
| C22:164n-3    |               |                        |             |             |
| C22:165n-3    |               |                        |             |             |
| C22:166n-3    |               |                        |             |             |
| C22:167n-3    |               |                        |             |             |
| C22:168n-3    |               |                        |             |             |
| C22:169n-3    |               |                        |             |             |
| C22:170n-3    |               |                        |             |             |
| C22:171n-3    |               |                        |             |             |
| C22:172n-3    |               |                        |             |             |
| C22:173n-3    |               |                        |             |             |
| C22:174n-3    |               |                        |             |             |
| C22:175n-3    |               |                        |             |             |
| C22:176n-3    |               |                        |             |             |
| C22:177n-3    |               |                        |             |             |
| C22:178n-3    |               |                        |             |             |
| C22:179n-3    |               |                        |             |             |
| C22:180n-3    |               |                        |             |             |
| C22:181n-3    |               |                        |             |             |
| C22:182n-3    |               |                        |             |             |
| C22:183n-3    |               |                        |             |             |
| C22:184n-3    |               |                        |             |             |
| C22:185n-3    |               |                        |             |             |
| C22:186n-3    |               |                        |             |             |
| C22:187n-3    |               |                        |             |             |
| C22:188n-3    |               |                        |             |             |
| C22:189n-3    |               |                        |             |             |
| C22:190n-3    |               |                        |             |             |
| C22:191n-3    |               |                        |             |             |
| C22:192n-3    |               |                        |             |             |
| C22:193n-3    |               |                        |             |             |
| C22:194n-3    |               |                        |             |             |
| C22:195n-3    |               |                        |             |             |
| C22:196n-3    |               |                        |             |             |
| C22:197n-3    |               |                        |             |             |
| C22:198n-3    |               |                        |             |             |
| C22:199n-3    |               |                        |             |             |
| C22:200n-3    |               |                        |             |             |
| C22:201n-3    |               |                        |             |             |
| C22:202n-3    |               |                        |             |             |
| C22:203n-3    |               |                        |             |             |
| C22:204n-3    |               |                        |             |             |
| C22:205n-3    |               |                        |             |             |
| C22:206n-3    |               |                        |             |             |
| C22:207n-3    |               |                        |             |             |
| C22:208n-3    |               |                        |             |             |
| C22:209n-3    |               |                        |             |             |
| C22:210n-3    |               |                        |             |             |
| C22:211n-3    |               |                        |             |             |
| C22:212n-3    |               |                        |             |             |
| C22:213n-3    |               |                        |             |             |
| C22:214n-3    |               |                        |             |             |
| C22:215n-3    |               |                        |             |             |
| C22:216n-3    |               |                        |             |             |
| C22:217n-3    |               |                        |             |             |
| C22:218n-3    |               |                        |             |             |
| C22:219n-3    |               |                        |             |             |
| C22:220n-3    |               |                        |             |             |
| C22:221n-3    |               |                        |             |             |
| C22:222n-3    |               |                        |             |             |
| C22:223n-3    |               |                        |             |             |
| C22:224n-3    |               |                        |             |             |
| C22:225n-3    |               |                        |             |             |
| C22:226n-3    |               |                        |             |             |
| C22:227n-3    |               |                        |             |             |
| C22:228n-3    |               |                        |             |             |
| C22:229n-3    |               |                        |             |             |
| C22:230n-3    |               |                        |             |             |
| C22:231n-3    |               |                        |             |             |
| C22:232n-3    |               |                        |             |             |
| C22:233n-3    |               |                        |             |             |
| C22:234n-3    |               |                        |             |             |
| C22:235n-3    |               |                        |             |             |
| C22:236n-3    |               |                        |             |             |
| C22:237n-3    |               |                        |             |             |
| C22:238n-3    |               |                        |             |             |
| C22:239n-3    |               |                        |             |             |
| C22:240n-3    |               |                        |             |             |
| C22:241n-3    |               |                        |             |             |
| C22:242n-3    |               |                        |             |             |
| C22:243n-3    |               |                        |             |             |
| C22:244n-3    |               |                        |             |             |
| C22:245n-3    |               |                        |             |             |
| C22:246n-3    |               |                        |             |             |
| C22:247n-3    |               |                        |             |             |
| C22:248n-3    |               |                        |             |             |
| C22:249n-3    |               |                        |             |             |
| C22:250n-3    |               |                        |             |             |
| C22:251n-3    |               |                        |             |             |
| C22:252n-3    |               |                        |             |             |
| C22:253n-3    |               |                        |             |             |
| C22:254n-3    |               |                        |             |             |
| C22:255n-3    |               |                        |             |             |
| C22:256n-3    |               |                        |             |             |
| C22:257n-3    |               |                        |             |             |
| C22:258n-3    |               |                        |             |             |
| C22:259n-3    |               |                        |             |             |
| C22:260n-3    |               |                        |             |             |
| C22:261n-3    |               |                        |             |             |
| C22:262n-3    |               |                        |             |             |
| C22:          |               |                        |             |             |

FIG. 64B

|         |     |   |     |
|---------|-----|---|-----|
| HSSEL01 | 208 | LQFVLTIQITSCGIVIMP-C-----TFPLGMLYFDI-GYMIISLIA    | 244 |
| CELELO  | 211 | QDFVLTITHTLSAVVKP-C-----GFPFGCLIFQS-SYMTLVI       | 247 |
| GGEL01  | 248 | TQFCMMSVQASSMDMVAAMKVLGRLGRGPFGYPF-FITALLWFMWTMLG | 289 |
| CELELO  | 218 | QDFIISCAVLLAHLYMLHTNANCDFEP5VFKLAVFMDTLYLA        | 260 |
| HSSEL01 | 245 | LETFNFEYDITYNKKGASRSRKDOLHDQNGHNSMAAVNGHTNSFSP    | 287 |
| GGEL01  | 248 | LFELNFYDITYRKKPV-----KKELDQ-----K-EVKNGFPKAHLLIV  | 282 |
| CELELO  | 290 | LFYNEFIDYQITDYLAKQAKADAAKEKARLKQ                  | 318 |
| HSSEL01 | 261 | LFVNFFDQLSYVLLRGKDKYKAVPKKKNN                     | 288 |
| CELELO  | 288 | ENNVKPRKLKD                                       | 299 |
| HSSEL01 | 283 | ANGMHTDKKAQ                                       | 292 |

FIG. 64A

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1 ACAGGGACT TTCTGTGA TTGTTGAG GCAAGAGAAG GAAAGATGGC  
 51 AACAGGAGC GTGTGGATG ATGTGGATG CGGGTGGAG ACCGGCTGG  
 101 ACCAGTGGAT GGAATGGCC AACGGCTACG CACTACCGA TGGGCTCCG  
 151 ATGATGGAG TGTCACCAT GCTGGATTC GAGGTGGAT ACATGGCCAT  
 201 GCTGCTTC GGCATCCCA GCAATGGAA GATGGAGAAG CCTTTGAGC  
 251 TCAGACCAT CAAGCTCTG CACAATTGT TTCTCTGG ACTTCCCTG  
 301 TAATGTGG TGAGACCAT CGCCAGGCT ATCCTCGAG GCTACAAAGT  
 351 GTTGGAAC GACAATGGAGA AGGGCAACGA GTCTCATGCT CAGGGCATGT  
 401 CTCGATGCT GTACGTGTT TACGTGTCCA AGGCATACGA GTTCTGGAT  
 451 ACCGGCATCA TGATCCTTG CAGAAGTTC AACAGGTTT CCTCTTGCA  
 501 TGTTACAC CAGGCACCA TTGTTGCCAT CTGGTGGCT ATGGCAAGT  
 551 ACCGTCAGG AGGTGATGG TACTTTAG TGTACCTAA CTCTTCATC  
 601 CAGTTATCA TGACTCTTA CTACCCCTGAA

FIG. 65

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1 TGDFLNLQ AQGRMANSS VWDVVMGAG KPYALTDGLP  
 51 MMDVSTMLAF EVGMMMLF GIPIMKQMEK PFEKTIKLL HNLFLFGSLI  
 101 YMCFETRQA ILGGYKFGN DMERGNESHA QGMSRIVVF YVSKAYEFLD  
 151 TAIMLCKKF NQVSFLHVVH HATIFAIWMA IAKYAPGGDA YFSVILNSFI  
 201 HWMYSSPE

FIG. 66

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1 ATGGCAAACA GCAGCGTGTG GGATGATGTG GTGGCCCGG TGGAGACCGG  
 51 CGTGGACCG TGGATGGATG GGCGCAAGC GTACCGCACTC ACCGATGGGC  
 101 TCCCGATGATG GGAGCTGTCC ACCATGCGTGG CATTGAGGT GGGATACATG  
 151 GCGCATGCTGC TCTTCGGCAT CCCGATCATG AAGCAGATGG AGAAGGCTTT  
 201 TGAAGCTCAAG ACCATCAAGC TCTTGACAA CTTGGACCTT CTCGGACCTT  
 251 CCTTGGTACAT GTGGGTGGAG ACCATCGGGC AGGCTATCCT CGGAGGCTAC  
 301 AAAGTGTGG GAAACGACAT GGAAGGGC AACGAGTCTC ATGCTCAGG  
 351 CATGTCGCA ATGTTGTAAG TGTTCGGT GTCCAAAGGCA TAGAGTTCT  
 401 TGGATACCGC CATCATGATC CTTTGCAGAA AGTTCAACCA AGTTCAACCA  
 451 TTGCAATGTT ACCACCATGC CACCATTTT GGCATCTGGT GGGCTATGCG  
 501 CAAAGTGTGG CAGGAGGTG ATGGCTACTT TTCAACTCTT CTCAACTCTT  
 551 TGGTGCACAC CGTCATGTAC GCATACACT TCTTCCTC CCAAGGGTTC  
 601 GGGTTGTGA AGCCAATCAA GCGGTACATC ACCACCTTC AGATGACCCA  
 651 GTTCAATGGCA ATGCTGTGC AGTCTGTGA CGACTACCTC TTCCCATGCG  
 701 ACTACCCACA GGCTCTGTG CAGCTCTGTG GAGTGTACAT GATCACCTTG  
 751 CTTGGCCCTCT TCGCAACTT TTTTGTGAG AGCTATCTTA AAAAGCCAA  
 801 AAAGAGCAAG ACCAACTAA

FIG. 67

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1 ATGCCAAACA GCAGGGTGTG GGATGATGTG GTGGGCCCGG TGGAGACCGG  
 51 CGTGGACCG TGGATGGATG GGCGCAAGC GTACGGACTC ACCGATGGGC  
 101 CCCCGATGATG GGACCTGTGC ACCATGCTGG CATTGAGGT GGGATACATG  
 151 GCGCATGCTGC TCTTCGGCAT CCCGATCATG AAGCAGATGG AGAAGGCTTT  
 201 TGAAGCTCAAG ACCATCAAGC TCTTGACAA CTTGGACCTT CTCGGACCTT  
 251 CCTTGGTACAT GTGGGTGGAG ACCATCGGGC AGGCTATCCT CGGAGGCTAC  
 301 AAAGTGTGG GAAACGACAT GGAAGGGC AACGAGTCTC ATGCTCAGG  
 351 CATGTCGCA ATGTTGTAAG TGTTCGGT GTCCAAAGGCA TAGAGTTCT  
 401 TGGATACCGC CATCATGATC CTTTGCAGAA AGTTCAACCA AGTTCAACCA  
 451 TTGCAATGTT ACCACCATGC CACCATTTT GGCATCTGGT GGGCTATGCG  
 501 CAAAGTGTGG CAGGAGGTG ATGGCTACTT TTCAACTCTT CTCAACTCTT  
 551 TGGTGCACAC CGTCATGTAC GCATACACT TCTTCCTC CCAAGGGTTC  
 601 GGGTTGTGA AGCCAATCAA GCGGTACATC ACCACCTTC AGATGACCCA  
 651 GTTCAATGGCA ATGCTGTGC AGTCTGTGA CGACTACCTC TTCCCATGCG  
 701 ACTACCCACA GGCTCTGTG CAGCTCTGTG GAGTGTACAT GATCACCTTG  
 751 CTTGGCCCTCT TCGCAACTT TTTTGTGAG AGCTATCTTA AAAAGCCAA  
 801 AAAGAGCAAG ACCAACTAA

FIG. 68

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1 ATGGAACAG CAGCGTGG GATGATGG TGGCCGGT GGAGACCAGC  
 51 GTGGACAGT GGATGGATG CGCAAGCC TAGCACTTA CGATGGCT  
 101 CCCGATGATG GACGTGCC CCGATGCC ATTGAGGTG GGATACATG  
 151 CCA TGCTGCT CTTCGGATC CGATCATGA AGCAGATGA GAAGGCTTT  
 201 GAGCTAAGA CATCAGCT CTTCGACAA TGTGTTCTCT TGGACTTC  
 251 CTGGTACATG TGGGTGAGA CATCCGCCA GGCTATCCC GGAGGTACA  
 301 AAGGTGTTGG AAGCGACAT GAGAGCTCA TGCCTGGC  
 351 ATGTCCTGCA TGTGTACGT GTTCTAGTG TCCAGGGAT AGAGGTTCTT  
 401 GGATACCGCC ATCATGATCC TTGCAAGAA GTTCAACCAAG GTTTCCTCT  
 451 TCGAAGTGTAA CCACCATGCC ACCATTTTG CATCTGGTG GGCTATGCC  
 501 AAGTACGCTC CAGGAGTGA TGGGTACATT TCAACTCTT  
 551 CGTGCACCC GTCATGTACG CATACTATT CTTCCTCC CAAGGGTTG  
 601 GGTTTGTAAG GCGAATCAAG CGTACATCA CACCCCTTA GATGACCCAG  
 651 TTATGGCAA TGCCTTGCA GTCCTGTAC GACTACCTCT TCCCATGGAA  
 701 CTACCCACAG GCTCTTGC AGCTCTGG AGTGTACATG ATCACCTTGC  
 751 TTGGCCCTCTT CGCGCAACTT TTGTGAGA GCTATCTAA AAAGCCAAA  
 801 AGAGGAAG CCAACTAA

FIG.69

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1 ATGGAACACA GCACCGTGTG GGATGATGTG GTGGCCCGCG TGAGACCGG  
 51 CGTGGACAG TGGATGGATG CGCGCAAGCC GTACGCACTC ACCGATGGGC  
 101 TCCGATGATG GACGTGCC ACCATGCTGG CATTGAGGTG GGGATACATG  
 151 GCGATCTGC TCTCGGAT CCCGATCATG AAGCAGATGA AGAAGCTTT  
 201 TGAAGCTCAAG ACCATCAAGC TCTTGCAAA CTTGGACTTC  
 251 CTTGTCACAT GTGGGTGAG ACCATCCGC AGGTATCCCT CGGAGGTAC  
 301 AAGGTGTTGG AAGCGACAT GAGAGCTCA TGCCTGGC  
 351 ATGTCCTGCA TGTGTACGT GTTCTAGTG GTCCAGGGCA AGTTCACCA GGTTTCCTC  
 401 TGGATACCGC CATCATGTC CTTGGCAGA AGTTCACCA GGTTTCCTC  
 451 TTGATGTGT ACCACATGC CACCGTTTT GCGCATCTGGT GGCGTATCGC  
 501 CAAAGTACGCTC CAGGAGTGA TGGGTACATT TCAACTCTT  
 551 TGTGTACAC CGTCAATGAC GCACTACTC TCTTCCTTC CAAGGGTTG  
 601 GGTTTGTAAG GCGAATCAAG CGTACATCA CACCCCTTA GATGACCCAG  
 651 GTTTCATGGCA ATGCTGTGC AGTCTTGTGA GACTACCTCT TCCCATGGAA  
 701 ACTACCCACA GAGCTCTGG CAGTTCTTG GAGTGTACAT GATCACCTTGC  
 751 CTGGCCCTCTT CGCGCAACTT TTGTGAGA GCTATCTAA AAAGCCAAA  
 801 AAAGGAAAG ACCAACTAA

FIG.70

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1 ATGGCAAAAC GCAAGCTGTG GATGGTGTG GTGGCCGG TGGAGACGG  
 51 CGTGGACAG TGGATGGAT GCGCAAGC GTACGGACTC ACCGAAGGC  
 101 TCCGGATGAT GGAGGTGTC ACCATGGCTT CATTGGAGT GGGATACATG  
 151 GCGATGTC TCTCGGGAT CCCATCATG AAGCAGATGG AGAAGGCTTT  
 201 TGAAGCTCAAG ACCATCAAG TCTTGACAA CTTGGACCTT  
 251 CCTTGATGACAT GTGGGTGGAG ACCATCCGGC AGGCTATCCG CGGAGGCTAC  
 301 AAAGTGTGG GAAACGACAT GAGAAAGGG AACGAGTCTC ATGCTAGGG  
 351 CATGTCGCG ATGSGTACG ATGCTAGT GTTCTCTAGT GTTCTAGT GTGAGTTCT  
 401 TGATACCGC CAATGATC CTTTGCAAGA AGTTCAACCA GGTTCCTTC  
 451 TTGCAATGCG ACCACATGC CACCATTTT GGGCATCTGG GGGCTATCGC  
 501 CAAGTACGCT CAGGAGGTG ATGGTACTT TTCAACTGTC CTCAACTCTT  
 551 TGGTGACAC CGTCATGTAC GCATACTACT TCTTCCTTC CCAAGGGTT  
 601 GGTTCTGTGA AGCCAATCAA GCGGTACATC ACCACCCCTC AGATGACCCA  
 651 GTTCATGGCA ATGCTGTGC ATGCTGTGA GAGTACCTC TTCCATGG  
 701 ACTACCCACA GGCTCTGTG CAGCTCTGT GAGTGTACAT GATCACCTG  
 751 CTTGGCCTCT TCGCAACTT TTTGTGAG AGCTATCTT AAAAGCAAA  
 801 AAAGGCAAG ACCAACTAA

FIG. 71

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1 ATGGCAAAAC GCAAGCTGTG GATGGTGTG GTGGCCGG TGGAGACGG  
 51 CGTGGACAG TGGATGGAT GCGCAAGC GTACGGACTC ACCGAAGGC  
 101 TCCGGATGAT GGAGGTGTC ACCATGGCTT CATTGGAGT GGGATACATG  
 151 GCGATGTC TCTCGGGAT CCCATCATG AAGCAGATGG AGAAGGCTTT  
 201 TGAAGCTCAAG ACCATCAAG TCTTGACAA CTTGGACCTT  
 251 CCTTGATGACAT GTGGGTGGAG ACCATCCGGC AGGCTATCCG CGGAGGCTAC  
 301 AAAGTGTGG GAAACGACAT GAGAAAGGG AACGAGTCTC ATGCTAGGG  
 351 CATGTCGCG ATGSGTACG ATGCTAGT GTTCTCTAGT GTTCTAGT GTGAGTTCT  
 401 TGATACCGC CAATGATC CTTTGCAAGA AGTTCAACCA GGTTCCTTC  
 451 TTGCAATGCG ACCACATGC CACCATTTT GGGCATCTGG GGGCTATCGC  
 501 CAAGTACGCT CAGGAGGTG ATGGTACTT TTCAACTGTC CTCAACTCTT  
 551 TGGTGACAC CGTCATGTAC GCATACTACT TCTTCCTTC CCAAGGGTT  
 601 GGTTCTGTGA AGCCAATCAA GCGGTACATC ACCACCCCTC AGATGACCCA  
 651 GTTCATGGCA ATGCTGTGC ATGCTGTGA GAGTACCTC TTCCATGG  
 701 ACTACCCACA GGCTCTGTG CAGCTCTGT GAGTGTACAT GATCACCTG  
 751 CTTGGCCTCT TCGCAACTT TTTGTGAG AGCTATCTT AAAAGCAAA  
 801 AAAGGCAAG ACCAACTAA

FIG. 72

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1 ATGGCAAACA GCAGGGTGTG GGATGATGTG GTGGGCCGCG TGGAGACCGG  
 51 CGGGACCA TGATGGATG GCGCAAGCC GTCAGCACTC ACCGATGGC  
 101 TCCCGATGAT GGACGTGTC ACATGCTGG CATTGAGGGT GGGATACATG  
 151 GCGATGCTGC TCTTCGGCAT ACCTGATCA TG AGGAGATGG AGGAGCCCTT  
 201 TGAATCAAG ACATCAAG TCTTGACAA CTGGTTCCTC TTGGACTCTT  
 251 CCTTGACAT GTGGGTGGT ACACATCCGC ACATCTCC TTGGAGGTAC  
 301 AAAGTGTGGT GAAAGCAGAT GGAGAAGGGC AAGGAGTCTC ATGCTCAGGG  
 351 CATGTCCTGC ATCGTGTAG TGTTCCTAGT GTCAAGGGAA TAGAGTTCT  
 401 TGGATACCGC CATCATGTC CTTTGCAAGA ATTCACACCA GGTTCCTTC  
 451 TTGATGTGT ACCACATGC CACCACTTTT GCGATCTGGT GGGCTATCGC  
 501 CAAGTAGCT CCAGGGGTG ATGGTACTT TTCACTGATC CTCACCTCTT  
 551 TCGTGACAC CGTCATGTC GCATACTACT TCTTCCTC CCAAGGGTTC  
 601 GGGTTCTGCA AGCCAACTAA GCGCTACATC ACCACCTTC AGATGACCA  
 651 GTTCATGGCA ATGCTTGTGC AGTCCCTGTA CGACTACCTC TTCCATGCG  
 701 ACTACCCACA GGCTCTTGTG CAGCTCTCTG GAGTGTACAT GATCACCTG  
 751 CTGGCCCTCT CGGCAACTT TTTTGCGAG AGCTATCTTA AAAAGCCAA  
 801 AAAGAGGAAG ACCAACTAA

FIG. 73

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1 MANSYYDDV VGRVETGVDDQ WMDGAKPVAL TDGLPMMDVS TMLADEVGYM  
 51 AMLLFGTIPIM KQMEKPFELK TIKLLHNFL EGLSLYMCVE TIRQA1LGGY  
 101 KIFGNDMEKG NESHAGQHMR IVYVFCVSKA YEFLOTAIMI LCKKFNIQVSF  
 151 LHVYHATIF AIWIAIAYKA PGGDAYFSV1 LNSFH1HTWYKAYYFFSSQGF  
 201 GFVKPIKPY1 TTLQMTQFMA MLVQSLYDYL FPCDYPQALV QLLGVYMTL  
 251 LAFLGNNFFVQ SYLKKPKSK TN\*

FIG. 74

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1 MANSVWDDV VGRVETGVQ WMDGAKPYAL TDGPPMDVS TMLAEFGYM  
 51 AMLFGIPIM KMEKPFELK TIKLLNLFL FGLSLYMCVE TIRQATLGYY  
 101 KVFGNDMEKG NESHAGMSR IIVAYFMSKA YEFDTAIMI LCKKFNQVSE  
 151 LHVYHATIF AIWAIAYKA PGDAYFSVI LNSFVHTWNY AYFFFSSQGF  
 201 GFVKPIKPYI TTLQMTQFMA MLVQSLYDYL FPCDYPQALV QLGVYMITL  
 251 LAFLGNNFFVQ SYLKKPKKSK TN\*

FIG. 75

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1 MATAACGMMW WAAMRPAANTS GIMMAPSRTHS PMGSR\*\*WTCP. PCMHSRWDTW  
 51 PCSSASRS\* SRMPSLSSR PSSCTTCS SDFPCTCAIR PSARLSSEAT  
 101 KCLETTMRRA TSLMLRACIA SCTCSTCRH TSSWIPPS\*S FARSSTRFPS  
 151 CKCTTRPPFL PSGGLSPSTL GEVMRTFQ\*S STLSCTPSCT HTTSSPPKGS  
 201 GS\*QSSRTS PPFR+PSSWQ CLCSPCTTS SHATRLLC SFLECT\*SPC  
 251 LPSSATEFLCR AILKSQRAR PT

FIG. 76

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1 MANSVWDDV VGRVETGVDDQ WMDGAKPYAL TDGLPMMDVS TMIAFEVGYM  
 51 AMLLFGIPIM KQNEKPFELK TIKLHNFL FGSLYMCVE TIRQATLGGY  
 101 KWFQNDMEKG NESHAGMSR IVYFYVSKA YEFLOTAIMI LCKKFQNVSF  
 151 LHVYHHTVF AIWIAIAKYA PGGDAYFSV1 LNSFVHTWVY AYFFSSQGF  
 201 GFVKPIKPY1 TTLQMTQFMA MLVQSLYDYL FPCDYPQALV QLLGVYMTL  
 251 LAIAGNFFVQ SYLKKPKSK TN\*

FIG. 77

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1 MANSSVWDDV VGRVETGVDDQ WMDGAKPYAL TDGLPMMDVS TMIAFEVGYM  
 51 AMLLFGIPIM KQNEKPFELK TIKLHNFL FGSLYMCVE TIRQATLGGY  
 101 KWFQNDMEKG NESHAGMSR IVYFYVSKA YEFLOTAIMI LCKKFQNVSF  
 151 LHVYHHTVF AIWIAIAKYA PGGDAYFSV1 LNSFVHTWVY AYFFSSQGF  
 201 GFVKPIKPY1 TTLQMTQFMA MLVQSLYDYL FPCDYPQALV QLLGVYMTL  
 251 LAIAGNFFVQ SYLKKPKSK TN\*

FIG. 78

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1 MANSVWDDV VGRVETGVDDQ WMDGAKPYAL TDGLPMMDVS TMIAFEVGYM  
 51 AMLLFGTIPIM K0MERPFELK TIKLLHNLFL FGSLYNCVE TIRQALIGGY  
 101 KVFGNDMEKG NESHAGMSR IVYVFVSKA YEFDTAIMI LCKKFNQVSF  
 151 LHVYHATIF AIWWAJAKYA PGDAYFSV1 LNSFVHTVMY AYYFFSSQGF  
 201 GFVKPIKPY1 TTLQMTQFMA MLVQSLYDYL FPCDYPQALV QLLGYM1TL  
 251 LAI FGNFFVQ SYLKPKKSK TN\*

FIG.79

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1 MANSVWDDV VGRVETGVDDQ WMDGAKPYAL TDGLPMMDVS TMIAFEVGYM  
 51 AMLLFGTIPIM K0MERPFELK TIKLLHNLFL FGSLYNCVE TIRQALIGGY  
 101 KVFGNDMEKG NESHAGMSR IVYVFVSKA YEFDTAIMI LCKKFNQVSF  
 151 LHVYHATIF AIWWAJAKYA PGDAYFSV1 LNSFVHTVMY AYYFFSSQGF  
 201 GFVKPIKPY1 TTLQMTQFMA MLVQSLYDYL FPCDYPQALV QLLGYM1TL  
 251 LAI FGNFFVQ SYLKPKKSK TN\*

FIG.80

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FIG.81A

\*% CONVERSION=PRODUCT/(SUBSTRATE+PRODUCT)

| % CONVERSION*   | 76.8%     | 28.4%     | 3.8%      | 82.2%     | 75.3%     | 81.5%     | 76.9%     |        |
|-----------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|--------|
| 334(PLASMID)    | PRAT-4-A1 | PRAT-4-A2 | PRAT-4-A3 | PRAT-4-A4 | PRAT-4-A6 | PRAT-4-A7 | PRAT-4-D1 | PRX242 |
| ADDED SUBSTRATE | GIA       |        |
| % TOTAL UDP     |           |           |           |           |           |           |           |        |
| C16:1n-7        | 16.95     | 5.22      | 15.52     | 11.06     | 14.49     | 15.69     | 15.18     | 11.24  |
| C18:0           | 4.95      | 6.73      | 5.94      | 4.17      | 4.57      | 4.25      | 4.79      | 5.64   |
| C18:1n-9        | 20.26     | 44.21     | 22.54     | 17.22     | 16.89     | 16.51     | 18.92     | 28.72  |
| C18:1n-7        | 2.87      | 1.64      | 2.97      | 7.51      | 1.85      | 3.90      | 3.50      | 1.70   |
| C18:2n-6        | 1.03      | 1.39      | 1.44      | 0.67      | 1.32      | 0.28      | 0.36      | 5.53   |
| C18:3n-6        | 3.05      | 1.09      | 5.28      | 0.67      | 2.38      | 1.89      | 2.45      | 2.79   |
| C20:3n-6        | 10.09     | 0.43      | 0.21      | 11.04     | 5.76      | 10.77     | 9.28      | 2.24   |
| C22:0           | 0.57      |           |           |           |           |           |           |        |

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FIG.81B

\*% CONVERSION=PRODUCT/(SUBSTRATE+PRODUCT)

| % CONVERSION*   | 1.7%      | 0.5%      | 7.6%      | 3.3%      | 12.4%     | 2.8%      |           |        |
|-----------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|--------|
| 334(PLASMID)    | PRAT-4-A1 | PRAT-4-A2 | PRAT-4-A3 | PRAT-4-A4 | PRAT-4-A6 | PRAT-4-A7 | PRAT-4-D1 | PRX242 |
| ADDED SUBSTRATE | EPA       |        |
| % TOTAL UDP     |           |           |           |           |           |           |           |        |
| C16:1n-7        | 18.35     | 17.55     | 7.96      | 11.80     | 14.06     | 11.53     | 9.68      | 20.66  |
| C18:0           | 3.41      | 3.69      | 2.57      | 2.90      | 3.18      | 3.10      | 3.20      | 8.49   |
| C18:1n-9        | 15.22     | 16.44     | 10.69     | 12.28     | 13.01     | 11.50     | 12.85     | 16.27  |
| C18:1n-7        | 1.36      | 2.12      | 1.60      | 5.62      | 2.13      | 2.02      | 2.35      | 1.33   |
| C18:2n-6        | 1.07      | 0.40      | 31.54     | 46.96     | 37.98     | 40.40     | 32.68     | 43.67  |
| C20:5n-3        | 23.24     | 0.40      | 0.16      | 0.09      | 0.12      | 0.09      | 0.12      | 0.83   |
| C22:1n-9        | 0.40      | 0.09      | 0.09      | 0.12      | 0.17      | 0.21      | 0.21      | 0.35   |
| C22:5n-3        | 0.40      | 0.40      | 0.40      | 0.40      | 0.40      | 0.40      | 0.40      | 0.40   |

FIG. 82

**SUBSTITUTE SHEET (BULE 26)**

FIG. 82B

SUBSTITUTE SHEET (B)(1) E 26

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 SCORES Init1: 259 Opt: 509 z-score: 86.5 E(): 0.28  
 >>EN:HSEL01  
 initn: 259 init1: 259 opt: 509 Z-score: 86.5 expect(): 0.28  
 Smith-Waterman score: 511; 34.0% identity in 265 aa overlap  
 (4-265:9-257)

|        |   |     |     |     |     |     |
|--------|---|-----|-----|-----|-----|-----|
| TEL01  | MANSSWDDIVGRVETGVDDWMDGAKPVALTDLGLPMDISTMLAFLETGYAMLLF    | 10  | 20  | 30  | 40  | 50  |
| HSEL01 | MEHFDASLSTYFALLGPDRTRVKGW.....FLLDNYIPTFICSVI.....YLLIWVL | 10  | 20  | 30  | 40  |     |
|        |   |     |     |     |     |     |
| TEL01  | GIPIMKQMEKPFELKTKLHNLEFGSLSYMCTVETRQALGGYKVFQNDIEKGNESHA  | 60  | 70  | 80  | 90  | 100 |
| HSEL01 | GPKVMRN-KQPFSRGILVWYNNGLTLLSLYMFCELVGKYNFFCQGTRTAGESDM    | 50  | 60  | 70  | 80  | 90  |
|        |   |     |     |     |     |     |
| TEL01  | QGMSRIVVVFVSKAYEFLDTAIMLCKKFNOVSFLHVYHATIFATWAKYAPGGDA    | 120 | 130 | 140 | 150 | 160 |
| HSEL01 | K-TIRVLWVYFSKLIEFMDTFFLTKNNHQITVLHVYHASNLTNWVNNWVPCGHS    | 110 | 120 | 130 | 140 | 150 |
|        |   |     |     |     |     |     |
| TEL01  | YFSVTLNSVWHTVWYAYFFSQGGFKPKI...KPYITTLQNTQFMAMLVSLYDLYFP  | 180 | 190 | 200 | 210 | 220 |
| HSEL01 | YFGATLNSFIHWLMSYGLSS...VPSMRPMLWKKRYTQGQLQFVLTITQTSGGVIMP | 170 | 180 | 190 | 200 | 210 |
|        |   |     |     |     |     |     |
| TEL01  | CDYQPALVQLLGIVMILLALFGNFFIOSYKKPKKSXTXNCLHDPLAGRIDSESEL   | 240 | 250 | 260 | 270 | 280 |
| HSEL01 | CTFPGLWYQIGMTSLIAFLTMFYQTYWKGASRRKDHLKHQNSMSAANVNGHTNSF   | 230 | 240 | 250 | 260 | 270 |
|        |   |     |     |     |     |     |

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 SCORES Init1: 278 Initn: 278 Opt: 515 z-score: 86.1 E(): 0.3  
 >>EN:MELO4  
 initn: 278 init1: 278 opt: 515 z-score: 86.1 expect(): 0.3  
 Smith-Waterman score: 517; 34.1% identity in 267 aa overlap  
 (8-270:16-255)

|       |  |     |     |     |     |     |
|-------|--|-----|-----|-----|-----|-----|
| TEL01 | MANSSWDDIVGRVETGVDDWMDGAKPVALTDLGLPMDISTMLAFLETGYAM        | 10  | 20  | 30  | 40  | 50  |
| MELO4 | MEQLKAFDNEVNAFLDNMFPRDOSRVGM.....FLDOSYLPT.....FLITITYL    | 10  | 20  | 30  |     |     |
|       |  |     |     |     |     |     |
| TEL01 | LLFGIPIMKQMEKPF-ELKTKLHNLFLLGFLSLYNCVETRQALGGYK/FGNDMEKG   | 60  | 70  | 80  | 90  | 100 |
| MELO4 | TIWLNKYMKN-RPAISLRGSLTLYNLATLTSAYMLVEILSSWEGGYNLQCQNLDSAG  | 50  | 60  | 70  | 80  | 90  |
|       |  |     |     |     |     |     |
| TEL01 | ESHAQGNSRIVVVFVSKAYEFLDTAIMLCKKFNOVSFLHVYHATIFATWAKYAP     | 120 | 130 | 140 | 150 | 160 |
| MELO4 | EGDVR-VAKVLWVYFSKLVEFLDTIFFVLRKTKTNQITFLHVYHASNLTNWVCLNWIP | 110 | 120 | 130 | 140 | 150 |
|       |  |     |     |     |     |     |
| TEL01 | GGDAYFSVTLNSFVHTWYAYFFSQGGFKPKI...-KPYITTLQNTQFMAMLVSLYD   | 180 | 190 | 200 | 210 | 220 |
| MELO4 | CGOSFFGPTLNSFIHWLMSYGLSV..FPMHMKYLWAKKYLTAQQLVQFLTITLTS    | 170 | 180 | 190 | 200 | 210 |
|       |  |     |     |     |     |     |
| TEL01 | YLFPCDYDPAVLQVLGIVMILLALFGNFFIOSYKKPKKSXTXNCLHDPLAGRID     | 230 | 240 | 250 | 260 | 270 |
| MELO4 | VVKPCGFPGCLIFQSSYMTLVILENFYQTYRKPKVKELEKEVKGPKANLIV        | 230 | 240 | 250 | 260 | 270 |
|       |  |     |     |     |     |     |

FIG.83

FIG.84

SCORES Init1: 412 Initn: 541 Opt: 628 z-score: 102.7 E(): 0.035  
 >>EN:GLELO  
 1initn: 541 1init1: 412 opt: 628 Z-score: 102.7 expect(): 0.035  
 Smith-Waterman score: 628; 43.4% identity in 244 aa overlap  
 (34-272:69-308)

10 20 30 40 50 60  
 TEL01 SSWDDVVGRVETGVQDMGAKPVYALTOGLPMDVSTMLAFAEVGIMAMLLFGIPIMKQM  
 GLELO LVAQAEKYIPTIVHHTRGFLVAVESPLAREPLMNPFLVLLIVLAYLVTIVFGMGIQMKNF  
 40 50 60 70 80 90

70 80 90 100 110 120  
 TEL01 EKPFEIJKTLLNLFGLSYMCEVETRQAIIGGKVKFGNDMEKGNESHAQGMSRIVY  
 GLELO ER-FEVKTFSSLNFCLVISIAYMCGGILYEAVQANYGLFENAAAD.-HTFKGLPMARNW  
 100 110 120 130 140 150

130 140 150 160 170 180  
 TEL01 VFYISKAYEFDLTAIMTLCKKFNQVSFLHVYHHATIFAIWAIKAYAPGGDAIFSVLNS  
 GLELO LFYSKIMFFVDTMIVLKKNRQTSFLHVYHHISSTIWNLVTFVAPNGEAYFSAALNS  
 160 170 180 190 200 210

190 200 210 220 230  
 TEL01 FVHTWYAYFFSSQGFVWPKPKVPTTQMTQFMANLVIOSLY-YLFPC...-DYPQA  
 GLELO FIHVIMGYFLSALGEKQWSFKEFYITRSQMTQFCMMSQSSDMYANKVLRGPFF  
 220 230 240 250 260

240 250 260 270 280 290  
 TEL01 LVQLLGVVMTLLALFGNFTIQSYLKKPKKSKTNXCLHDPLAGYRIDSSELRHANS  
 GLELO ITALLMFYHMTMLGLFNMFYRKNA-KLAQAKADAKEKARKLQ  
 280 290 300 310

98/102 SCORES Init1: 94 Initn: 220 Opt: 302 z-score: 61.6 E(): 6.5  
 >>EN:CEELO  
 1initn: 220 1init1: 94 opt: 302 Z-score: 61.6 expect(): 6.5  
 Smith-Waterman score: 312; 34.3% identity in 239 aa overlap  
 (49-270:54-278)

10 20 30 40 50 60 70  
 TEL01 DQWMDGAKPVYALTDLGPMMDVSTMLAFAEVGIMAMLLFGIPIMKQMKEPKFELKTIKL-LHN  
 GLELO ATHGPKNFDPDAEGRKFFAAHFDVTDIQAISILW-WVVFGRKWFMRNRPFPQL-TIPLNWN  
 30 40 50 60 70 80

80 90 100 110 120  
 TEL01 LFIFGLSLYMCVE.....TI-ROAIIQGY-KVFGNDMEKGNESHAQGMSRIVYFYVS  
 GLELO FILAASFJAGAKWMTPEFFGTIAKGVIVASCKIF-.DFTKGENGYM.....VNLFMAS  
 90 100 110 120 130

130 140 150 160 170 180  
 TEL01 KAYEFLDTAIMTLCKKFNQVSFLHVYHHATIFAIWAIKAYAPGGDAYFSVTLNSFVHTV  
 GLELO KLFELVDITFLVLRKR-.PMFLHWWHMHILTMITYAWYSHPLTPGFNRY-GIYLNFVNHAF  
 140 150 160 170 180 190

190 200 210 220 230 240  
 TEL01 MTAAYFFSSQGFVWPKPKVPTTQMTQFMANLVIOSLY-YLFPC...-DYPQA  
 GLELO MTSYFYFLSKWIKRIPGFLIAQATSLQIVOF1ISCIAVLAHLGYLMHFTNANDFEPFSVFKL  
 200 210 220 230 240 250

250 260 270 280 290 300  
 TEL01 LGYVM-ITLLALFGNFTIQSYLKKPKKSKTNXCLHDPLAGYRIDSSELRHANSIFF  
 GLELO -AIFMDDTYALWFNFYHLSYURGGDQKTKAVPKKKNN  
 260 270 280

FIG. 85

FIG. 86

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| 334(PLASMID)            | pRAT-4-A4 | pRAT-4-A6 | pRAT-4-A7 | pRAT-4-D1 | pYX242 | pRAT-4-A4 | pRAT-4-A6 | pRAT-4-A7 | pRAT-4-D1 | pYX242 | pRAT-4-A4 | pRAT-4-A6 | pRAT-4-A7 | pRAT-4-D1 | pYX242 |
|-------------------------|-----------|-----------|-----------|-----------|--------|-----------|-----------|-----------|-----------|--------|-----------|-----------|-----------|-----------|--------|
| ADDED SUBSTRATE         | GLA       | GLA       | GLA       | GLA       | GLA    | AA        | AA        | AA        | AA        | AA     | STA       | STA       | STA       | STA       | STA    |
| % TOTAL LIPID           |           |           |           |           |        |           |           |           |           |        |           |           |           |           |        |
| C16:1n-7                | 36.29     | 39.18     | 38.58     | 38.84     | 45.68  | 30.36     | 35.19     | 33.47     | 33.70     | 45.06  | 34.43     | 39.63     | 38.03     | 38.74     | 45.94  |
| C18:0                   | 2.78      | 3.13      | 2.73      | 3.05      | 1.76   | 2.51      | 2.78      | 2.56      | 2.72      | 1.65   | 4.01      | 3.13      | 3.06      | 3.76      | 1.82   |
| C18:1n-9                | 15.89     | 16.55     | 15.92     | 16.34     | 13.23  | 14.60     | 15.14     | 15.39     | 15.26     | 13.01  | 16.05     | 16.51     | 17.37     | 19.07     | 14.10  |
| C18:1w7                 | 5.91      | 2.01      | 3.44      | 2.06      | 0.93   | 5.72      | 1.94      | 3.75      | 2.22      | 1.03   | 4.92      | 1.78      | 3.78      | 2.52      | 0.95   |
| C18:3w6                 | 2.10      | 1.96      | 1.60      | 2.19      | 5.29   |           |           |           |           |        | 0.83      | 0.88      | 0.53      | 1.09      | 3.42   |
| C18:4w3                 |           |           |           |           |        |           |           |           |           |        |           |           |           |           |        |
| C20:3n6                 | 4.56      | 4.23      | 5.42      | 3.88      |        |           |           |           |           | 0.04   |           |           |           |           |        |
| C20:4w6                 |           |           |           |           |        | 16.53     | 13.61     | 14.21     | 17.13     | 7.54   |           |           |           |           |        |
| C20:4w3                 |           |           |           |           |        |           |           |           |           |        | 2.27      | 3.25      | 3.95      | 4.01      | 0.06   |
| C22:4w6                 |           |           |           |           |        | 0.57      | 0.20      | 0.75      | 0.18      |        |           |           |           |           |        |
| C22:4n-3                |           |           |           |           |        |           |           |           |           |        | 1.10      | 0.19      | 0.63      | 0.16      |        |
| % CONVERSION*<br>OF C18 | 68.4%     | 68.4%     | 77.2%     | 64.0%     |        |           |           |           |           |        | 80.3%     | 79.6%     | 89.7%     | 79.3%     |        |
| % CONVERSION*<br>OF C20 |           |           |           |           |        | 3.4%      | 1.5%      | 5.0%      | 1.0%      |        | 32.6%     | 5.5%      | 13.8%     | 3.8%      |        |

FIG.87A

## SUBSTITUTE SHEET (RULE 26)

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| 334(PLASMID)    | pRAT-4-A4 | pRAT-4-A6 | pRAT-4-A7 | pRAT-4-D1 | pYX242 | pRAT-4-A4 | pRAT-4-A6 | pRAT-4-A7 | pRAT-4-D1 | pYX242 |  |  |  |  |  |
|-----------------|-----------|-----------|-----------|-----------|--------|-----------|-----------|-----------|-----------|--------|--|--|--|--|--|
| ADDED SUBSTRATE | EPA       | EPA       | EPA       | EPA       | EPA    | NONE      | NONE      | NONE      | NONE      | NONE   |  |  |  |  |  |
| % TOTAL LIPID   |           |           |           |           |        |           |           |           |           |        |  |  |  |  |  |
| C16:1w7         | 31.51     | 33.92     | 34.31     | 34.74     | 47.12  | 39.54     | 41.51     | 41.44     | 43.02     | 46.45  |  |  |  |  |  |
| C18:0           | 2.76      | 3.09      | 2.78      | 2.77      | 1.79   | 2.45      | 3.21      | 2.59      | 3.07      | 2.05   |  |  |  |  |  |
| C18:1w9         | 15.69     | 15.79     | 16.49     | 15.22     | 13.95  | 18.56     | 19.08     | 17.98     | 18.80     | 14.03  |  |  |  |  |  |
| C18:1w7         | 6.07      | 1.97      | 3.72      | 1.89      | 1.05   | 8.77      | 2.57      | 4.86      | 2.77      | 1.07   |  |  |  |  |  |
| C20:1w7         | 0.22      | 0.08      | 0.13      | 0.07      |        | 0.61      | 0.07      | 0.14      | 0.07      |        |  |  |  |  |  |
| C20:5w3         | 11.88     | 15.20     | 11.32     | 13.91     | 6.41   |           |           |           |           |        |  |  |  |  |  |
| C22:5n-3        | 1.49      | 0.54      | 1.74      | 0.54      |        |           |           |           |           |        |  |  |  |  |  |
| % CONVERSION*   | 11.1%     | 3.4%      | 13.3%     | 3.7%      |        |           |           |           |           |        |  |  |  |  |  |

\*% CONVERSION=PRODUCT/(SUBSTRATE+PRODUCT)

FIG.87B

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SUBSTITUTE SHEET (RULE 26)

| 334(PLASMID)         | pYX242 | pRAT-4-A7 |
|----------------------|--------|-----------|--------|-----------|--------|-----------|--------|-----------|--------|-----------|--------|-----------|--------|-----------|--------|-----------|
| ADDED SUBSTRATE      | LA     | LA        | ALA    | ALA       | GLA    | GLA       | STA    | STA       | DGLA   | DGLA      | ETA    | ETA       | AA     | AA        | EPA    | EPA       |
| % TOTAL LIPID        |        |           |        |           |        |           |        |           |        |           |        |           |        |           |        |           |
| C16:1n-7             | 38.73  | 27.71     | 39.05  | 26.97     | 35.28  | 30.13     | 34.75  | 30.95     | 35.96  | 28.51     | 34.91  | 31.45     | 34.71  | 24.76     | 40.02  | 24.75     |
| C16:1n-5             | 0.37   | 0.17      | 0.32   | 0.20      | 0.25   | 0.21      | 0.27   | 0.18      | 0.33   | 0.22      | 0.31   | 0.21      | 0.32   | 0.20      | 0.40   | 0.21      |
| C18:0                | 2.21   | 2.80      | 2.14   | 3.27      | 2.12   | 3.14      | 2.44   | 3.28      | 2.15   | 2.86      | 2.55   | 3.52      | 2.42   | 2.71      | 2.75   | 3.26      |
| C18:1n-9             | 11.14  | 12.79     | 11.73  | 15.73     | 10.14  | 14.24     | 11.13  | 14.79     | 11.17  | 14.59     | 11.33  | 16.32     | 11.63  | 13.85     | 12.86  | 15.99     |
| C18:1n-7             | 0.85   | 3.19      | 0.73   | 3.84      | 0.64   | 3.31      | 0.78   | 3.01      | 0.96   | 3.71      | 0.95   | 3.89      | 1.06   | 3.49      | 1.11   | 3.87      |
| C18:2n-6             | 3.04   | 3.54      |        |           |        |           |        |           |        |           |        |           |        |           |        |           |
| C18:3n-6             |        |           |        |           | 4.49   | 1.59      |        |           |        |           |        |           |        |           |        |           |
| C18:3n-3             |        |           | 3.55   | 3.56      |        |           |        |           |        |           |        |           |        |           |        |           |
| C18:4n-3             |        |           |        |           |        |           | 3.09   | 0.85      |        |           |        |           |        |           |        |           |
| C20:1n-7             |        | 0.10      |        |           | 0.09   |           | 0.24   |           | 0.24   |           | 0.09   |           | 0.10   |           | 0.22   | 0.27      |
| C20:2n-6             |        |           | 1.07   |           |        |           |        |           |        |           | 0.08   |           |        |           |        |           |
| C20:3n-6             |        |           |        |           |        | 5.84      |        |           |        | 9.08      | 14.13  |           |        |           | 0.06   |           |
| C20:4n-6             |        |           |        |           |        |           |        |           |        |           |        |           |        | 14.60     | 20.89  |           |
| C20:3n-3             |        |           |        | 2.71      |        |           |        |           |        | 0.10      |        |           |        |           |        |           |
| C20:4n-3             |        |           |        |           |        |           |        | 4.19      |        |           | 5.06   | 8.49      |        |           |        |           |
| C20:5n-3             |        |           |        |           |        |           |        |           |        |           | 0.16   | 0.25      |        |           | 6.47   | 15.32     |
| C22:4n-7             |        |           |        |           | 0.11   | 0.19      | 0.14   | 0.18      | 0.13   | 0.14      | 0.13   | 0.24      | 0.14   | 0.22      |        | 0.27      |
| C22:4n-6             |        |           |        |           |        |           |        |           |        |           |        |           |        |           | 0.76   |           |
| C22:4n-3             |        |           |        |           |        |           |        |           | 0.74   |           |        |           | 1.04   |           |        |           |
| C22:5n-3             |        |           |        |           |        |           |        |           |        |           |        |           |        |           |        | 1.99      |
| % CONVERSION* OF C18 | 23.2%  |           | 43.3%  |           | 78.6%  |           | 85.3%  |           |        |           |        |           |        |           |        |           |
| % CONVERSION OF C20  |        |           |        |           |        |           | 15.0%  |           |        |           |        | 10.9%     |        | 3.5%      |        | 11.5%     |

\*% CONVERSION=PRODUCT/(SUBSTRATE+PRODUCT)

FIG.88

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